

Ecological effects of *Ulva lactuca* L. in Avon-Heathcote Estuary

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Abstract

Macroalgal blooms are increasing world-wide and have negative effects on benthic invertebrates and sediments. These include loss of species diversity and development of hostile sediment environments. This thesis considers ecological effects of *Ulva lactuca* L., and its mechanical removal on benthic invertebrates and sediments in Avon-Heathcote Estuary, New Zealand. Benthic communities comprised 34 species from 12 groups recorded from seven sites during seasonal general surveys. Dominant groups at each site were Gastropoda and Bivalvia. The most abundant species were *Austrovenus stutchburyi*, *Micrelenchus tenebrosus* and *Amphibola crenata*. Community composition varied significantly between sites, and there were significant site-specific differences in abundances of most species between winter and summer. *U. lactuca* had the greatest seasonal variation. Several species correlated with *U. lactuca* biomass, and the strength of correlation for different species varied between sites. There were seasonal changes in sediment physico-chemical variables between sites with greatest change in the silt/clay fraction. The sediment variables silt/clay fraction, dissolved oxygen and temperature correlated with seasonal changes of patterns in benthic community assemblages. A similar study was carried out by Bressington in 2003. In both studies, Bivalvia and Gastropoda were the most abundant groups, with Gastropoda having a higher, and Bivalvia a lower, proportion in the present study compared with 2003. Summer communities were significantly different between the two studies. Compared with 2003 there were higher percentages of sediment pore water and volatile solids present in 2005.

Experimental removal of *U. lactuca* was conducted by mechanical broom at two sites: an open, exposed central sand flat, and McCormacks Bay, a shallow, sheltered mud flat. Removing *U. lactuca* had several immediate effects. These included a significant decrease in abundance of mobile epifauna (*Micrelenchus tenebrosus* and *Zeacumantus subcarinatus*) and an increase in abundance of infauna, including *Arthritica bifurca* and *Austrovenus stutchburyi*. There was no effect of *U. lactuca* removal on *Austrovenus stutchburyi* condition and 46 days following removal, invertebrate abundances approached pre-removal levels at each site. *U. lactuca* removal also caused short-term increases in dissolved oxygen and temperature of pore water. The greatest visual impact of removing *U. lactuca* was to sediments in McCormacks Bay from trampling.

It was concluded that the variables having the greatest effect on seasonal species' distribution and abundance at each site were temperature and sediment grain size. Differences between the present study and the study in 2003 were due to differences in sampling procedure mainly due to the two different quadrat sizes. Greater accuracy in representing long-term changes in ecosystems would be achieved by using standard sampling protocols. Removal of *U. lactuca* by mechanical broom was effective and had low impact on benthic invertebrates and physico-chemical variables, but it should be used only in sandy habitats because of severe disturbance to soft-sediment environments. Options for management and control of *U. lactuca* in Avon-Heathcote Estuary are discussed.

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Chapter 1

Introduction to estuarine ecosystems and Avon-Heathcote Estuary

1.1 Introduction

Estuaries are important global transition zones between freshwater and marine environments (Day 1981; McLusky 1981; Perillo 1995; Robertson *et al.* 2002). An estuary has been defined as: “a semi-enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage” (Pritchard 1967). However, Day’s (1980) amended definition: “An estuary is a partially enclosed body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixing of sea water with fresh water derived from land drainage”, encompasses estuaries in arid countries that become temporarily hypersaline during periods of elevated temperatures in summer, or are temporarily cut off from the sea during the dry season.

Estuaries are fragile ecosystems with a high diversity of habitats and include rocky outcrops, sandy beaches, mangroves and salt marshes, and subtidal to intertidal areas (Day 1981; Raffaelli 1992; Robertson *et al.* 2002; Paerl *et al.* 2003). They support a wide range of permanent flora and fauna including phytoplankton, macroalgae, seagrasses, diverse invertebrates, fish, and birds, and are environments often visited by migratory birds and pelagic fish that use these areas as feeding grounds and nurseries (Knox and Kilner 1973; Day 1981; Owen 1992). These ecosystems are subjected to continual stress from human habitation of the immediate area and their surrounding watersheds (Meyer-Reil and Koster 2000; GESAMP 2001; Robertson *et al.* 2002). Stressors include eutrophication, heavy metal contamination, hydrologic manipulations, aquaculture, fishing harvest, exotic species and climate change (Nixon 1995; Cloern 2001; Oreja and Salinas 2003; Rosales-Hoz *et al.* 2003; Cardoso *et al.* 2004a; Zhou *et al.* 2004). Eutrophication and heavy metal contamination are results of disposing industrial and urban waste products that in large quantities overburden natural recycling mechanisms of the system (Steffensen 1974; Oreja and Salinas 2003). The

products are either transported by riverine systems or directly discharged into estuaries where they accumulate (Steffensen 1974; Oreja and Salinas 2003). These then manifest multiple negative ecological impacts on estuaries such as loss of habitat quality and biodiversity, an overall increase in primary production, and macroalgal blooms (Hull 1987; Schramm and Nienhuis 1996; Paerl 1997; Schramm 1999; Cardoso *et al.* 2004a).

Estuary classification

Estuaries are as complex as they are diverse (Dyer 1979; Fairbridge 1980; Perillo 1995; Hume 2003). Their morphological features have been and are continually influenced by geological processes such as: tectonic activity, erosion, transportation and deposition of sediment particles originating from terrestrial and marine environments (Schubel 1971; Dyer 1979; McLusky 1981; Perillo 1995). Estuaries are also subjected to diverse tidal conditions, fluvial hydrology, wind and wave action (Day 1981; Perillo 1995; Raffaelli *et al.* 1999). Many estuary classification schemes have been submitted in the past. Pritchard (1952) presented the first geomorphologic classification that included drowned river valleys, bar-built estuaries and fjords. Tectonic estuaries were then added to this classification by Pritchard (1960). Following this, Hayes (1975) classified estuaries by tidal range: microtidal <2 m, mesotidal 2-4 m, and macrotidal >4 m. Fairbridge (1980) classified estuaries in seven categories based on both physiographic and hydrodynamic factors. Dalrymple *et al.* (1992) produced a classification model based on combining river discharge, tides and waves with time. This was defined as an evolutionary classification by Perillo (1995). Later, he combined all the categories to develop a morphogenetic classification.

In New Zealand, there is a broad range of estuarine systems with approximately 300 being listed by McLay (1976). A classification scheme for this range was developed by Hume and Herdendorf (1988); estuaries were grouped into five classes that reflect primary processes that shaped their basin. Further subdivisions within each class were then made on a morphologic basis reflecting catchment and coastal hydrologic and sedimentologic processes. This resulted in 16 types of estuaries (Table 1).

Table 1.1: The classification developed for New Zealand estuaries by Hume and Herdendorf (1988) based on their origin, hydrology and geomorphology.

| Primary Mode of origin of depositional basin | Estuary type | |
|--|-------------------------|------------------------|
| Fluvial erosion | Funnel-shaped | Type 1 |
| | Headland enclosed | Type 2 |
| | Barrier enclosed | Double-spit Type 3 |
| | | Single-spit Type 4 |
| | | Tombolo Type 5 |
| | | Island Type 6 |
| | | Beach Type 7 |
| | River mouth | Straight-banked Type 8 |
| | | Spit-lagoon Type 9 |
| | | Spit-lagoon Type 10 |
| | | Deltaic Type 11 |
| Marine/fluvial | Coastal embayment | Type 12 |
| Tectonism | Fault defined embayment | Type 13 |
| | Diastrophic embayment | Type 14 |
| Volcanism | Volcanic embayment | Type 15 |
| Glaciation | Glacial embayment | Type 16 |

Eutrophication – cause and effects

Eutrophication is the process by which the increasing external input of nutrients, particularly mineral macronutrients (nitrogen and phosphorus), to a body of water increases its nutritional status (Nixon 1995; Richardson and Jorgensen 1996; Meyer-Reil and Koster 2000). Over the last century this has increased along with the expanding human population (Meyer-Reil and Koster 2000). Anthropogenic eutrophication is widely recognised as a major global pollution threat to coastal waters and estuaries (Knox and Kilner 1973; Nixon 1995; Fletcher 1996; Romero *et al.* 1996; Sfriso and Marcomini 1996; GESAMP 2001; Bricker *et al.* 2003). This has become a serious problem particularly because more than 70% of the world's human population lives in watersheds that drain to these areas (Baden *et al.* 1990;

Fletcher 1996; Peckol and Rivers 1996; Vitousek *et al.* 1997). Some examples are: Palmones River Estuary, Southern Spain (Hernandez *et al.* 1997; Vergara *et al.* 1997), Ythan Estuary, Aberdeenshire, Scotland (Raffaelli *et al.* 1999), Famosa Slough Estuary, San Diego, California, U.S.A. (Fong 2000), Fraser River estuary, Canada (Arvai *et al.* 2002), and Pearl River Delta, China (Fung *et al.* 2005). Nutrient enrichment results in notable primary and secondary symptoms of eutrophication (Fig. 1.1).

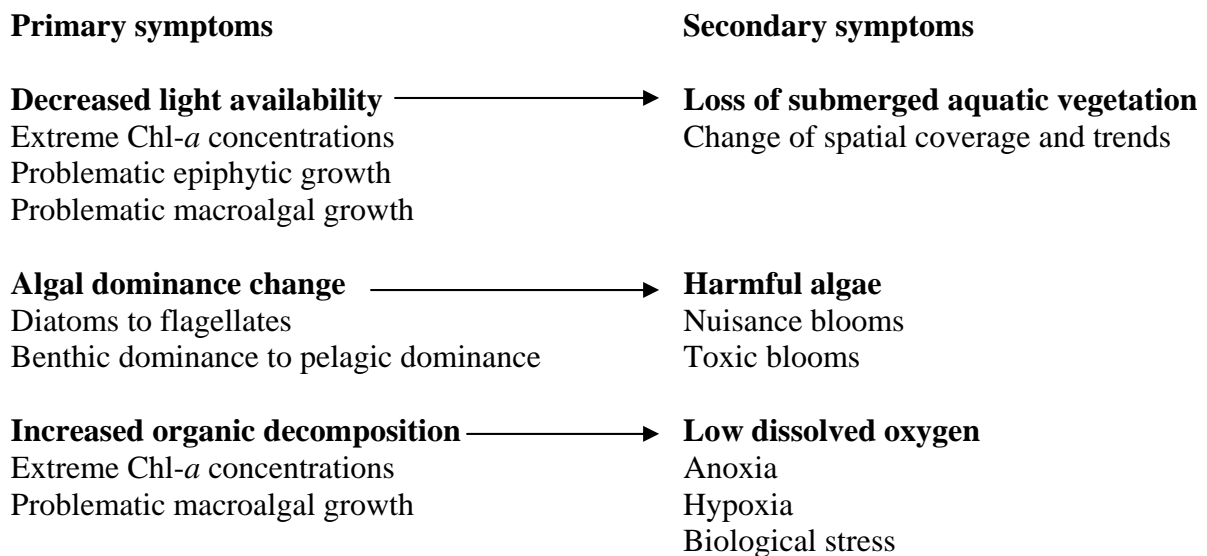


Figure 1.1: Primary and secondary symptoms of eutrophication in estuaries influenced by external inputs of nitrogen and phosphorus. From Bricker *et al.* (2003).

Nixon (1995) proposed four categories to characterise trophic status of marine coastal and estuarine ecosystems: oligotrophic, mesotrophic, eutrophic, and hypertrophic. These are defined by increasing rates of primary production: <100, 100-300, 301-500, and >500 g carbon m⁻² yr⁻¹ respectively. Historically, assessment of eutrophication in estuaries has been quantified by using the classical freshwater approach (Table 1.2 early phase I). However, this is not suitable for estuarine systems because nutrient concentrations are not a robust diagnostic variable; low levels do not indicate absence of eutrophication and high levels do not indicate eutrophication (Cloern 2001; Bricker *et al.* 2003). The contemporary approach (Phase II-Table 1.2) incorporates the complexity of direct and indirect scale-dependent interactions and responses that occur in estuarine systems (Raffaelli *et al.* 1998). This model was designed because advances in coastal research had identified key differences in the

response of lakes and coastal-estuarine ecosystems to nutrient enrichment (Cloern 2001; Bricker *et al.* 2003).

Table 1.2: Comparison of the conceptual Early Phase I¹ and Contemporary Phase II² models of coastal-estuarine eutrophication and the responses to nitrogen loading and heavy metal contamination. Adapted from Cloern (2001).

| Early Phase I Conceptual Model | | | |
|--|--|---|--|
| Effect | Response | | |
| Nutrient loading | Changes in:chlorophyll, primary production, system metabolism, dissolved oxygen, and nutrients (results in the establishment of a nutrient-based classification system). | | |
| Contemporary Phase II Conceptual Model | | | |
| Effect | Filter | Direct response | Indirect response |
| Nutrient loading. | Sensitive system attributes of an individual ecosystem act to modulate responses. These include inherent physical and biological factors such as tidal energy and freshwater inflow. | Changes in: chlorophyll, primary production, macroalgal biomass, sedimentation of organic C, Si, and N:P ratios, toxic algal blooms, phytoplankton and sub-tidal communities. | Changes in: benthos biomass and community, vascular plants, habitat quality/diversity, water clarity, organic C in sediments, sediment biogeochemistry, bottom-water dissolved oxygen, seasonal cycles, mortality of fish and invertebrates, nutrient cycling, and food web structure. |
| Heavy metal Contamination | | | |

¹ based on freshwater approach.

² adapted for coastal eutrophication.

Heavy metal contamination

Heavy metals such as zinc (Zn), copper (Cu), nickel (Ni), and aluminium (Al) occur naturally in silt- and clay-bearing minerals (Deely 1991; Oreja and Salinas 2003; Rosales-Hoz *et al.* 2003). However, industrial waste discharges cause changes in sediment dynamics and increased inputs of both hydrocarbons and heavy metals in estuarine sediments around the world (Saiz-Salinas 1997; Oreja and Salinas 2003; Rosales-Hoz *et al.* 2003; Zhou *et al.* 2004). Estuarine environments act as sinks for these fine grained contaminated-reactive sediments which accumulate, causing a significant long-term contamination problem (Deely 1991; Rosales-Hoz *et al.* 2003). This is because reworking of these sediments by dredging, land claim activities, and early diagenetic release of heavy metals cause continued inputs to the ecosystem (Cundy *et al.* 2003; Oreja and Salinas 2003; Rosales-Hoz *et al.* 2003).

Heavy metal contamination was documented by Rosales-Hoz *et al.* (2003) in Coatzacoalscos River and Estuary. This is a major petrochemical production centre and the most polluted coastal area of Mexico. They found elevated concentrations of a wide range of

heavy metals including Zn, Cu, Cr, Ni, and Al in the estuarine sediments compared with sediments upstream of the industrial area. Pearl River Estuary, the largest in southern China comprising 8000 km², is one of the country's greatest industrial and most densely populated areas (Zhou *et al.* 2004). Zhou *et al.* (2004) carried out experiments to determine distribution, source and enrichment of some chemical elements in sediments. Results showed distribution was mainly affected by grain size and type, and resuspension was an important factor in redistribution. Both these studies concluded that heavy metal contamination resulted in the degradation of environment quality and loss of habitat for flora and fauna, and contaminants are persistent even after cessation of, or decreases in, industrial discharge.

Macroalgal blooms

Increasing dominance of opportunistic green macroalgal blooms on intertidal flats and shallow sublittoral locations of the world's coastlines are well-documented (Fletcher 1996; Valiela *et al.* 1997; Raffaelli *et al.* 1998). These are a direct result of excessive nutrient availability, especially nitrogen and phosphorus (Schramm and Nienhuis 1996; Schramm 1999; Cloern 2001; Bricker *et al.* 2003) (Fig 1.1; Table 1.2). Macroalgae, such as *Ulva* spp., are physiologically resilient to stress from wide ranging light and salinity (Raffaelli *et al.* 1998; Schramm 1999), and growth on an area of shore is often limited only by availability of suitable substrate (Raffaelli *et al.* 1998). Blooms generally form dense beds in mid to low tide zones of estuaries and generate hostile physico-chemical environments in underlying sediments (Soulsby *et al.* 1982; Raffaelli *et al.* 1991; Norkko and Bonsdorff 1996a; Raffaelli *et al.* 1998; Raffaelli 2000; Bressington 2003). Detrimental effects of this include: burrowing bivalves being forced to the surface, exclusion of surface deposit feeders, and invertebrates declining in abundance (Murias *et al.* 1996; Norkko and Bonsdorff 1996a; Raffaelli *et al.* 1998; Lopes *et al.* 2000; Raffaelli 2000). Macroalgae present a significant nuisance when they detach, wash ashore, and decompose, as underlying sediments turn anoxic and sulphate-reducing bacteria produce toxic concentrations of unpleasant smelling hydrogen sulphide gas (Soulsby *et al.* 1982; Sfriso *et al.* 1987; Owen 1992; Fletcher 1996; Valiela *et al.* 1997; Modig and Olafsson 1998; Raffaelli *et al.* 1998; Bressington 2003).

Estuarine biota

Estuarine systems link marine and freshwater environments and therefore have a wide variety of habitats (Schubel 1971; Hume and Herdendorf 1988; Cowan and Boynton 1996; Hume 2003). Distinct assemblages of species are restricted to particular areas (Jones 1976; Blanchard and Bourget 1999; Edgar and Barrett 2002). Stenohaline marine species (those that are able to tolerate only a narrow range of salinity) are bound to areas close to the estuary mouth (Day 1981); these include crabs such as *Petrolisthes elongates* (Jones 1976) and fish such as mackerel and cod. Euryhaline marine species (those that can withstand a wide range of saline levels) can distribute throughout estuarine systems (Day 1981). These include species of crab and migratory fish such as salmon and whitebait. Freshwater species are bound by areas of very low salinity (Knox and Kilner 1973). These include fish such as brown trout and invertebrates such as larvae of stone flies and mayflies.

Estuaries support a diverse but limited range of species making up functional feeding groups that play an important role in the general functionality of the system (Raffaelli 1999; Osterling and Pihl 2001). These groups include: epibenthic predators including shore birds such as oystercatchers and plovers (Nicholls *et al.* 1981), whelks and fish such as flounders (Jones and Marsden 2005), grazers such as the mudflat snail *Amphibola crenata* (endemic to New Zealand) filter feeders such as the mussel *Mytilus edulis galloprovincialis*, suspension feeders including the cockle *Austrovenus stutchburyi*, deposit feeders including polychaetes such as *Capitella* spp., and primary producers such as the macroalgae *Ulva* spp. and species of microalgae (Knox and Kilner 1973; Cane 1996; Drake and Arias 1996; Raffaelli 1999; Jones and Marsden 2005). All of these groups are common in estuaries around the world (Morton and Miller 1973). One of the most diverse groups is the invertebrates. This group is made up of many species that can be further divided into epifauna (those normally living on the sediment surface and include decapods, gastropods and chitons) and infauna (those living below the sediment surface including bivalves, and polychaetes) (Knox and Kilner 1973). These estuarine species contribute to local assemblages that vary over spatial and temporal scales (Thrush *et al.* 1989; Thrush 1991; Thrush *et al.* 1994). They are often limited in distribution by their physiological tolerance to variations in environmental factors including: salinity, temperature, dissolved oxygen, substrate and exposure. The most important factor – salinity - forms gradients that are driven by mixing of marine and fresh water (Estcourt 1967; Knox and Kilner 1973; Bonsdorff *et al.* 1995; Blanchard and Bourget 1999).

1.2 Study System

Avon-Heathcote Estuary

Avon-Heathcote Estuary, Christchurch, South Island, New Zealand (43° 32' S, 172° 43' E) is a shallow (average depth ~ 1.4 m), fluvial erosion, semi-enclosed, bar-built estuary (Hume and Herdendorf 1988) of approximately 8 km² (Fig. 1.2 & Fig. 1.3). It is well flushed with the majority of total average tidal and freshwater inflow (~8.3 x 10⁶ m³) draining with each ebbing tide (Knox and Kilner 1973). The tides are microtidal (dominated by wind and wave action) (Perillo 1995); these are semi-diurnal with a maximum range of 1.7 m at neap and 2.2 m at spring tides (Knox and Kilner 1973). Approximately 85% of the Estuary is intertidal sand and mudflats. During low tide these are completely uncovered and exposed for longer periods with distance from the Estuary mouth. Exceptions are areas of standing water and river channels (Knox and Kilner 1973).

On calm days during incoming tides, a modified salt wedge with vertical stratification develops in these channels. Denser salt water flows up channel beneath the freshwater, forming two layers. These layers gradually mix as water flows out over intertidal flats (Jolly 1971; Knox and Kilner 1973). Normally the Estuary is prone to strong vertical mixing due to combinations of shallow depth, strong tidal action and wind (Estcourt 1962; Knox and Kilner 1973). At high tide, salt water penetrates Avon and Heathcote Rivers 8 and 11 km respectively upstream from the Estuary mouth. These rivers are the main freshwater inputs, spring fed, slow flowing and meandering (Knox and Kilner 1973). Avon River flows through residential, commercial, and rural areas and Heathcote River flows through residential and industrial areas. The rivers' catchments, including tributaries and drains, are approximately 188 km² (Knox and Kilner 1973; Stephenson 1981; Robertson *et al.* 2002). The major land use is prime pastoral and urban (Robertson *et al.* 2002) (Table 1.3) with a population of 344,100 (as of June 2004) (Bolton-Ritchie and Main 2005). Presently, the main point source discharge of contaminants is from the ~160,000 m³/day, with a consent allowing up to 500,000 m³/day, of treated municipal sewage released 1 hour before and 3 hours after high tide from the Christchurch Waste-water Treatment Plant's oxidation ponds (Bolton-Ritchie and Main 2005). Lincoln Environmental (1994) conducted tracer studies that indicated 45% of discharged waste-water returned with the following incoming tide.

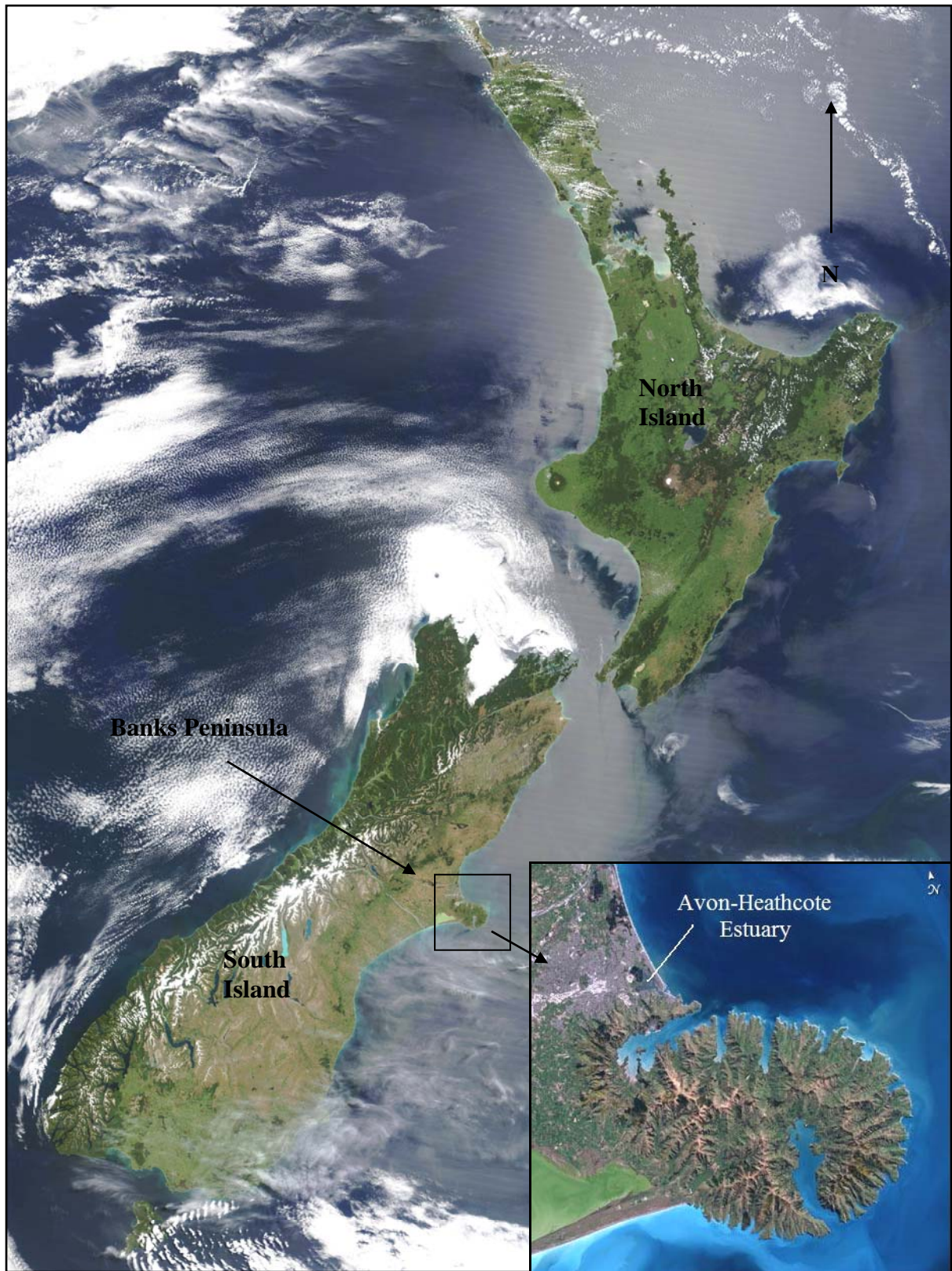


Figure 1.2: Satellite images of the North and South Islands of New Zealand. Insert image is of Banks Peninsula showing the location of the Avon-Heathcote Estuary.

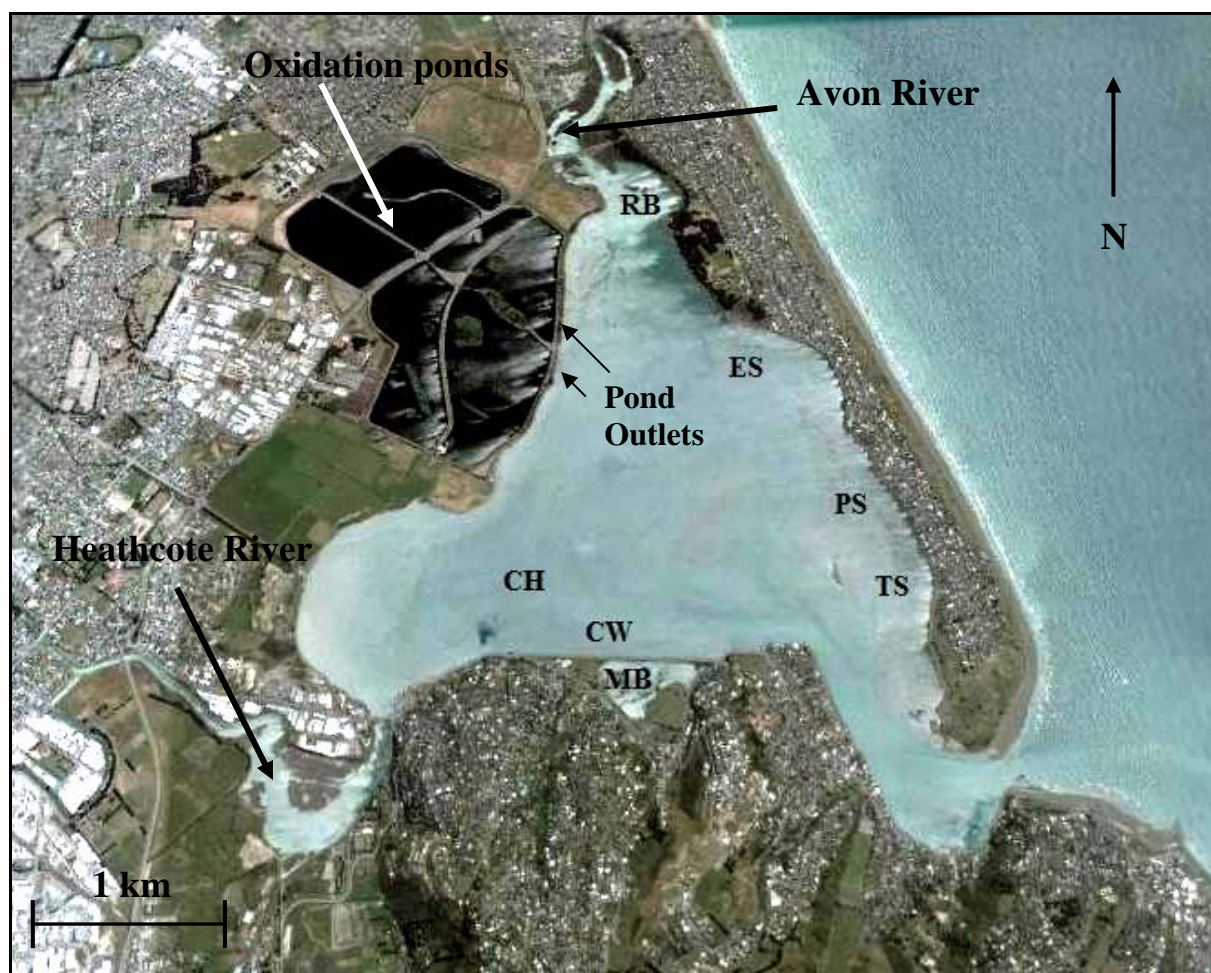


Figure 1.3: Satellite image of Avon-Heathcote Estuary showing locations of study sites, Causeway (CW), Channel (CH), Raupo Bay (RB), Ebb Tide Street (ES), Penguin Street (PS), Tern Street (TS) and McCormacks Bay (MB), entry of Avon and Heathcote Rivers, waste treatment works oxidation ponds, and pond outlets.

Table 1.3: Catchment land uses of the Avon-Heathcote Estuary. Adapted from (Robertson *et al.* 2002)

| Land use | Area (ha) | Cover (%) |
|---------------------|-----------|-----------|
| Urban | 10512.0 | 56.0 |
| Prime pastoral | 5050.6 | 26.9 |
| Urban open space | 1225.1 | 6.5 |
| Tussock | 591.6 | 3.2 |
| Planted forest | 579.2 | 3.1 |
| Inland water | 322.6 | 1.7 |
| Scrub | 276.5 | 1.5 |
| Prime horticultural | 189.3 | 1.0 |
| Indigenous forest | 22.7 | 0.1 |
| Bare ground | 13.1 | 0.1 |

Historic point source contaminants

Historically point source contaminants were in high concentrations (Knox and Kilner 1973; Robertson *et al.* 2002). Sewage effluent was piped to and released within the Estuary from the sewage farm constructed in 1882. This was constructed in response to the rapidly increasing population (Knox and Kilner 1973). The sewage farm was superseded in 1962 by the Christchurch Waste-water Treatment Plant, operating a two-stage treatment system. This included settling tanks, trickling filters and biological oxidation in a series of ponds (Knox and Kilner 1973; Owen 1992). These were completed in 1971, with upgrades in 1978, 1996, and 2004 (Knox and Kilner 1973; Bolton-Ritchie and Main 2005). Prior to 1965, sewage from communities bordering the southern shores was emptied directly into the Estuary from large septic tanks. From 1970 these and other areas were linked to Christchurch Waste-water Treatment Plant (Steffensen 1974). Between 1929 and 1980 the average daily flow of effluent into the Estuary increased from ~30.66 million litres to ~132.47 million litres per day (Knox and Kilner 1973). By 2001 it was estimated that effluent discharge from the oxidation ponds contributed to 90% of nitrogen and 98% of phosphorus input to the estuary (Bolton-Ritchie and Main 2005).

Industrial effluents were transported via the Heathcote River to the Estuary until 1970 when an industrial sewer was connected to the treatment works. Prior to this, 10 million litres a day of toxic untreated industrial effluent were discharged from ~150 industries directly into the Heathcote. The major industries were: woollen mills, tanneries, rubber factories, gelatine and glue factories, engineering works, a battery factory, wool scouring works and a fellmongery. These industries disposed of heavy metals and chemicals such as: sulphur, arsenic, chromium, hydrochloric acid, other acids, copper, lead, zinc, nickel, iron, and bisulphate of lime (Knox and Kilner 1973; Millward 1975; Owen 1992). In addition, heavy metals leached from the city dump, and spillage of petroleum products such as tar and oil were frequent and illegal dumping of these pollutants to the rivers was a common practice. Both rivers receive urban runoff and storm water inputs from many drains which add to the pollution problem (Knox and Kilner 1973; Steffensen 1974; Owen 1992; Robertson *et al.* 2002).

1.3 Objectives

Whilst harvesting is used to manage accumulations of macroalgae in eutrophic estuaries throughout the world, few studies have addressed the effects of harvesting on estuarine intertidal mudflats. The objectives of this thesis are: to examine how removing the dominant, mat-forming macroalgae, *Ulva lactuca* L, affects local community structure and physico-chemical variables of these habitats. General surveys were also conducted at selected sites (see section 2.2) to determine the seasonal changes in community assemblages and physico-chemical variables, and a comparison was made from a previous study in 2003 to determine longer temporal variations in community assemblages and physico-chemical variables.

The remainder of this thesis is organised as follows: Chapter 2 describes the study sites, main study species, statistical techniques, and general methodology used throughout the thesis. The results and discussion on the preliminary studies are also presented. Chapter 3 presents results and discussion of the investigation into seasonal changes in community structure and physico-chemical variables. Chapter 4 examines effects on benthic community structure and physico-chemical variables following the removal of *Ulva Lactuca* L from two intertidal mudflats; results and discussion are presented. Chapter 5 is the general discussion.

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(Pritchard 1952; Pritchard 1960; Hayes 1975; Mclay 1976; Dalrymple *et al.* 1992; Lincoln Environmental 1994)

(Norkko and Bonsdorff 1996b; Cardoso *et al.* 2004b)

Chapter 2

Study sites, general methodology and preliminary studies

2.1 Introduction

Quantitative measurements in ecology provide data that are used to define abiotic and biotic patterns and processes affecting distribution and abundance of biological assemblages (Kingsford and Battershill 1998; Quinn and Keough 2002). Obtaining efficient (in terms of costs) and precise representation of this is determined by sample size and number of replicates chosen (Quinn and Keough 2002). Increasing number of replicates leads to greater precision: standard error ($S.E.=s/\sqrt{n}$) divided by mean of the sample ($S.E./\bar{x}$) (Green 1979; Kingsford and Battershill 1998; Quinn and Keough 2002). This is influenced by understanding spatial and temporal distributions of organisms. Variance (s^2) /mean (\bar{x}) ratio is a good indication of whether a species is randomly dispersed ($v/m = 1$), is more clumped ($v/m > 1$) or is more ordered ($v/m < 1$) (McIntyre 1984). Also, knowledge of physico-chemical factors within study areas, results from previous studies, and conducting a preliminary survey contributes to understanding of these patterns and processes (Green 1979; Jongman *et al.* 1995; Fowler *et al.* 1998; Kingsford and Battershill 1998; Quinn and Keough 2002).

Preliminary observations in the Avon-Heathcote Estuary indicated that benthic fauna such as the mud flat snail (*Amphibola crenata*) and the top shell (*Micrelenchus tenebrosus*) displayed a clumped and limited distribution. Lewis and Taylor (1968) define this as the most common condition. Clumping of estuarine fauna is associated with limited dispersal (Lewis and Taylor 1986), spatial heterogeneity of habitats (Bonsdorff *et al.* 1995), and a combination of variable gradients in environmental factors (Estcourt 1967). These include: salinity, substrate and substrate availability, dissolved oxygen and temperature with the most pronounced factor thought to be salinity (Knox and Kilner 1973; Owen 1992).

Objectives of the preliminary studies were to determine: (1) appropriate quadrat size and number of replicates needed to reduce sampling error; (2) practicality of the experimental design including time required to collect and process all samples, and (3) compare sediment particle analysis and *Ulva lactuca* removal methods.

2.2 Study sites

McCormacks Bay (MB)

McCormacks Bay (Fig 2.1) is a highly modified, shallow, sheltered embayment located on the southern shore of the Estuary (Fig 1.3). The bay was modified in 1907 when the causeway was built across the tidal mudflats by the Tramway Board. Skylark Island, on the Estuary side, started to erode immediately after this. By 1922 it was reduced to mudflats. In 1933 the causeway was widened for motor traffic (Findlay and Kirk 1988). The Bay is currently connected to the main body of the Estuary by one large and two small culverts passing through the causeway. The causeway and surrounding banks of the Bay act as artificial rocky shores. Prolific beds of *Ulva lactuca* thrive in the optimal conditions of the Bay. The mudflats are gently sloping from the west past the man-made islands until reaching the main culvert channel (Fig 2.1). The mudflats retain surface water in shallow pools for the duration of low tide. An artificial reef projecting from the left side of the main culvert prevents the eastern area from draining at low tide. The east side of the artificial islands marks the extent of low tide in the western area.



Figure 2.1: Satellite image of McCormacks Bay showing the position of the study site, main culvert and islands.

Causeway (CW)

Site CW is located directly opposite McCormack Bay (Fig. 1.3). High tide mark is on rocks and cement used to construct the causeway. Intertidal sand and mudflats extend for ~20 m before dropping down into Heathcote channel which permanently retains water. The exposed flats are well drained with intermittent shallow pools.

Channel (CH)

Site CH (Fig. 1.3) is located on a slightly elevated dense cockle bed beside Heathcote channel. This site remains exposed for up to 4 hours depending on tide height. It is exposed at times to high prevailing winds and wave action.

Raupo Bay (RB)

Site RB (Fig. 1.3) is sheltered on three sides by mature stands of pine trees and fringed by wetland. Mudflats extend to Avon channel west of the Bay. These are exposed for long periods compared to other parts of the Estuary.

Ebb Tide Street (ES)

This site is flanked by a seawall on the Brighton sand spit (Fig. 1.3). The wall is an artificial high tide mark. Intertidal sandflats slope evenly and extensively to the low tide channel formed by the discharge from Avon River. These flats are open and exposed with a few large areas of shallow standing water.

Penguin Street (PS)

This site is adjacent to South Brighton sand spit (Fig. 1.3) where residential properties border the Estuary shore. The shore line maintains a low profile which has been modified with cobbles and boulders. Sea grass (*Zostera novazelandica*) dominates mid and low tidal areas. Sand and mudflats gently undulate to the Avon channel. Areas of standing water are a common occurrence.

Tern Street (TS)

This site is similar to Penguin Street with exception of denser *Z. novazelandica* meadows and a raised sandflat in mid tide zone (Fig. 1.3).

2.3 Main Study Species

Ulva lactuca L. (sea lettuce)

Ulva lactuca (division Chlorophyta, family Ulvaceae) (Lobban and Wynne 1981) is a naturally occurring, ephemeral, opportunistic green macroalga widespread throughout the world's marine shallow-water, intertidal embayments and estuaries (Raffaelli *et al.* 1998; Bolam *et al.* 2000; Cardoso *et al.* 2004b). *U. lactuca* are restricted to below mid-tide zone because of desiccation from long term exposure to the elements (Lobban *et al.* 1985). They are either attached to shells and other available hard substrate, or found as epiphytes on sea grass (*Zostera* sp.) and other species of macroalgae (Steffensen 1974; Knox 1986).

U. lactuca grow up to ~30 cm high, the thallus is broad (exceeding 1 meter if attached to large stable objects) with rounded lobes and smooth undulating margins without denticulations (Steffensen 1974; Adams 1994; Van Den Hoek *et al.* 1995). The thallus is two cell layers thick (cells are almost square) with a narrow division between layers (Steffensen 1974; Van Den Hoek 1981; Adams 1994; Van Den Hoek *et al.* 1995). The large surface area per unit volume is useful for intercepting incident light and nutrients (such as nitrogen and phosphorus) in large quantities when temporarily available. *U. lactuca* take up nutrients 4-6 times faster than slower growing perennial species (Pedersen and Borum 1997). This results in fast production of new biomass (Littler and Littler 1980; Carpenter 1990; Rivers and Peckol 1995; Raffaelli *et al.* 1998; Altamirano *et al.* 2000). With simple thallus morphology, each cell is exposed to the bathing medium, therefore cells can osmoregulate individually and adjust rapidly in response to tidal changes in osmolarity (Black and Weeks 1972; Young *et al.* 1987). These species are widely recognised as being stress-resistant; plants tolerate and exploit fluctuating and high temperatures associated with shallow water environments (Littler and Littler 1980; Rivers and Peckol 1995; Raffaelli *et al.* 1998). However, the thallus is easily shredded by shifting sediment and other particles. *Ulva* spp. lost 95.6% of the thallus during a wave shearing experiment (Littler and Littler 1980).

Reproduction is by biflagellate gametes or quadriflagellate zoospores on plants of similar form; male or female isogametes are produced on separate plants. Distinctive pale patches remain where fertile cells have shed their contents (Adams 1994; Van Den Hoek *et al.* 1995). Reproduction takes place in late winter/early spring resulting in a peak biomass during summer (Steffensen 1974). Germination is stimulated to a great extent by high light, low temperature, and increased nutrient supply (Schramm 1999).

Austrovenus stutchburyi (cockle)

The endemic little-neck clam *Austrovenus stutchburyi* is a dominant member of mid to low-tide estuarine and other soft shore communities in New Zealand (Morton and Miller 1973; Pilkington 1992; Marsden and Pilkington 1995; Marsden 2004). Population densities have been recorded up to $\sim 3000\text{m}^{-2}$ with individuals reaching 20 years old and shell lengths greater than 60 mm (Stephenson and Chanley 1979). When mature (18 – 20 mm independent of age) males and females spawn simultaneously. This occurs once a year in summer (Stephenson 1981; Pilkington 1992). *A. stutchburyi* is a sedentary burrower (Pilkington 1992) and reaches depths ranging from 2 to 4 cm (Knox and Kilner 1973). Once submerged by incoming tides these filter feeders extend their siphons to the sediment surface and continue to feed until shortly before being exposed by ebbing tides (Stephenson 1981; Pilkington 1992).

Estuarine environmental factors such as salinity, temperature, sediment type and food availability potentially influence the physiological condition of these bivalves (Stephenson 1981; Pilkington 1992; Marsden 2004). Furthermore, macroalgal mats cause anoxia of underlying sediments (Soulsby *et al.* 1982; Raffaelli *et al.* 1998; Bressington 2003) which has been shown to affect bivalve species by interfering with their feeding behaviour and altering food availability (Raffaelli *et al.* 1998). This may affect the health of the bivalves.

Condition indices are used to assess bivalve health (Crosby and Gale 1990; Pilkington 1992) and have been used to determine seasonal and site variation in the health of *A. stutchburyi* (Marsden and Pilkington 1995; Marsden 2004). Crosby and Gale (1990) provide a review of index methodologies and recommend a standard to be used; $\text{C.I.} = (\text{dry soft tissue wt (g)} \times 1000) / \text{internal shell cavity capacity (g)}$. However, for this study the condition index used by Pilkington (1992), Marsden (2004) and Maclaren (2005) ($\text{C.I.}_{\text{shell}} = (\text{dry tissue weight/shell dry weight}) \times 100$ (Crosby and Gale 1990) was selected. This is thought to be a reliable stress indicator for bivalves (Rainer and Mann 1992; Marsden and Pilkington 1995; Marsden 2004).

2.4 Statistical Techniques

This section describes statistical techniques used in this and following chapters. Data were analysed using a combination of univariate and multivariate analyses. Nonmetric Multi-Dimensional Scaling (MDS) ordination was used in PRIMER v6.1.5 to examine the effect of macroalgal removal on community composition. Community data were averaged (\bar{x}) for each plot from each site. Means (\bar{x}) were square-root-transformed to scale down effects of very abundant species (Clarke and Warwick 2001). MDS places samples on the ordination based upon their biotic similarity using Bray-Curtis similarity coefficients. MDS has no axis scores. Distances separating samples represent relative dissimilarity between sample groups (Clarke and Warwick 2001). MDS was also used on square-root-transformed invertebrate abundance data from all sites to define their dissimilarity. The displayed stress level in each MDS plot depicts the accuracy of the graphical representation of data; lower stress levels reflect higher accuracy. If stress >0.2, the representation is considered inaccurate (Clarke and Warwick 2001).

Relationships between biotic and environmental variables were investigated using BIOENV procedure of PRIMER v6.1.5. This calculates weighted Spearman rank correlation coefficients between the dissimilarity matrix underlying MDS (biotic) and Euclidean distance (environmental) matrices for all combinations of environmental variables and indicates which individual or set of habitat attributes best explains the measured biological patterns. The level of correlation is shown by the Rho-value (rank correlation coefficient) closer to 1 = high correlation, closer to 0 = low correlation (Clarke and Warwick 2001).

Principal components analysis (PCA) was used in PRIMER v6.1.5 to analyse environmental variables. This procedure is appropriate for environmental data as these have a complex mix of measurement scales such as pore water (%), salinity (ppt), and dissolved oxygen (mg/l^{-1}). The data were transformed and normalised resulting in comparable dimensionless scales. Greater accuracy of the true relationship between samples or sites is represented by a higher percentage of variation accounted for by the PC1 and PC2 axes. (Clarke and Warwick 2001).

Similarity of percentage (SIMPER), is a non-statistical method that shows a species contribution to the separation between two groups of samples, or the closeness of samples within a group (Clarke and Warwick 2001). This was used to determine which species contributed most to observed differences between undisturbed and treated plots.

Analysis of similarity (ANOSIM) was used to test the null hypothesis that differences did not occur in community composition and physico-chemical variables between sites, treatments and time of sampling. This test uses randomised permutations and the rank-similarity Bray-Curtis matrix to calculate the tests statistic (R) and significance level for differences between compositions of sample groups. The R-statistic normally lies between 0 and +1, where 0 indicates between sample similarity and 1 between sample dissimilarity. A negative value indicates greater within sample variability than between sample variability (Clarke 1993; Clarke and Warwick 2001). Interaction terms cannot be tested with ANOSIM. Therefore, sampling periods were analysed separately with the R-value used as an indicator of dissimilarity between times of treatments. Community data were standardised and square-root-transformed and physico-chemical data were square-root-transformed and normalised.

Analysis of variance (ANOVA) were calculated using SPSS v 11.0 and is a method of testing the null hypothesis that several group means are equal in the population (Dytham 1999). This is done by comparing the sample variance estimated from the group means to that estimated within the groups. ANOVAs were used to test for significant variations between sites and treatment samples. Data were tested for normality using the Kolmogorov-Smirnov test in SPSS v11.0, and where appropriate were square-root-transformed and normalised to fit the assumptions of ANOVA.

Pearson's correlation coefficients between the species data and each of the physical variables were calculated in SPSS v 11.0 after testing data for normality using the Kolmogorov-Smirnov test in SPSS v 11.0. When an increase in one variable is accompanied by an increase in another, the correlation is said to be positive. When an increase in one variable is accompanied by a decrease in another, the correlation is said to be negative (Quinn and Keough 2002).

2.5 Assessment of methods

Quadrat and sample size

Previous studies used quadrats measuring 15 x 15 cm (Bressington 2003), 25 x 25 cm (Robertson *et al.*, 2002) and 32 x 32 cm (Griffin and Thomson 1992). Based on findings by Bressington (2003) and the sampling protocol developed by Robertson *et al.* (2002), a comparison was made between the 15 x 15 cm and 25 x 25 cm quadrats. This was to determine the size and number of quadrats required to obtain practical representations of community structure and abundance. In December 2004, twenty of each size were randomly placed within mid-tide zone between sites RB and ES (Fig. 1.3). Sediment, including all benthic invertebrates, within each quadrat was removed to a depth of 5 cm and placed in

labelled plastic bags. In the laboratory, samples were wet sieved using a 1000 μm mesh and all species, excluding polychaetes, were identified and counted.

The mean (\bar{x}) and variance to mean ratio (s^2/\bar{x}) of all animals from both quadrat sizes were calculated. The mean was scaled to 1m^2 to compare the differences in species abundance between quadrat sizes. The precision (S.E./mean ratio) of sampling was compared in 20% confidence intervals between both quadrat sizes for *Amphibola crenata* and *Austrovenus stutchburyi*. These were the two most abundant species. Low S.E./mean ratios represent high precision (precision increases with sample size while the standard error is reduced). In Excel each quadrat was represented by a column. For both species and quadrat sizes precision was calculated cumulatively for the 20 quadrats. This was repeated 20 times after the quadrats had been rearranged at random. The minimum and maximum readings of precision from each of the 20 columns were plotted to show a comparison between quadrat sizes.

Sediment particle analysis

A comparison was made between traditional sieve and pipette methods, and the computer run Saturn DigiSizer 5200TM. This was conducted with three whole sediment samples and a single class of sediment from a previous study in Avon-Heathcote Estuary. These had been processed traditionally and stored in sealed envelopes by the Department of Geology, University of Canterbury, Christchurch, New Zealand. Samples were from different sites with different sediment profiles (S6 and S8) and distance from the high tide mark (S6A was closest to the shore and S6D was furthest from the shore). Data was square-root-transformed and normalised before analysis.

*Removal of *Ulva lactuca* mats*

Time trials were conducted at site CH to determine the most practical and efficient method of removing *Ulva lactuca* from an intertidal mudflat. Two methods were trialled: a mechanical hand held broom (driven by a 35cc petrol motor) with a rotating soft finned rubber attachment (Fig. 2.2), and a hand held 14-prong garden rake. One 2 x 8 m plot was cleared of *U. lactuca* by each method. Observations were made on the practicality and effectiveness of each method, and time taken to clear plots.



Figure 2.2: The rotating head attachment of the mechanical broom. This is attached to a 35 cc petrol driven scrub cutter assemblage and is normally used for sweeping large areas of gravel during road laying and maintenance.

2.6 Preliminary study and *Ulva lactuca*. removal experiment

Experimental design

Twelve 8 x 2 m permanent plots (Fig. 2.3) were established in February 2005 at an intertidal mudflat covered with *U. lactuca* in McCormacks Bay (in March 2005 the same design of plots was established at the Channel site for the purpose of the removal experiment see Chapter 4). Plots were located in mid tide zone at the North West side of the Bay (Fig. 2.1). All plots had an original cover of *U. Lactuca* spp. between 75% and 100%. Plots were separated by three meters from each other (Fig. 2.3). The corners of each plot were marked with wooden stakes. These plots were to be used in the preliminary survey and removal experiment. Sheets representing each plot were divided into 256 sub plots (8 across by 32 deep) each representing an area of 0.0625 m². Random numbers between 1 and 256 were generated for each plot and the first four numbers were marked on the sheets. These were used in the field to: (1) position each quadrat, (2) prevent pseudoreplication in future sampling by using subsequent numbers and (3) select an alternative placement when disturbance by trampling was encountered; this would help avoid incorrect organic measurements from the

sediment samples caused by algae that was trodden into the sediment. Samples of benthic invertebrates and physico-chemical variables were measured in a preliminary study from this site to determine the practical aspects of sampling between tides and the order of the processing of samples in the laboratory.

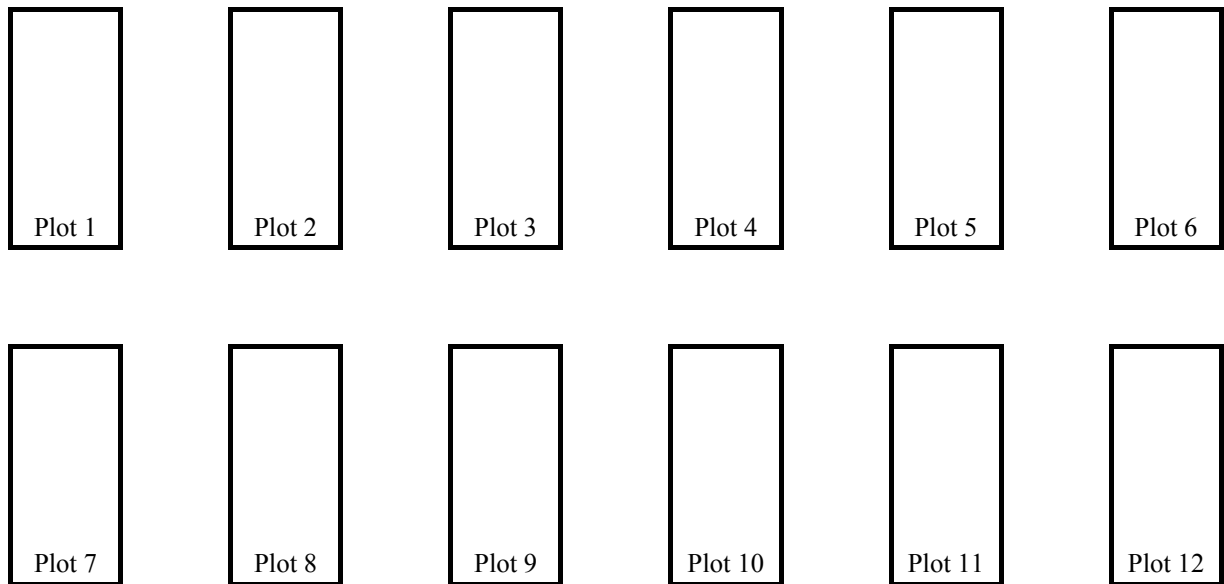


Figure 2.3: The layout of experimental plots used at the McCormacks Bay and Channel sites during the removal experiment.

2.7 General methodology (all studies)

Benthic invertebrates and macroalgae

Four 25 x 25 x 5 cm deep quadrats ($\sim 3125 \text{ cm}^3$) were inserted into the sediment. Solid sides prevented *H. crassa* from escaping deep into their burrows and excluded nearby animals falling into samples along with oozing sediment. Sediment including invertebrates and macroalgae was removed and placed in labelled plastic bags. In the laboratory samples were wet sieved using a 1000 μm mesh to separate invertebrates and macroalgae from sediment. Macroalgae were separated from invertebrates by floatation and rinsed to remove remaining invertebrates. Macroalgae were separated into species and placed on WettexTM absorbent cloths to remove surface water. Following this they were weighed to the nearest 0.01 g. They were then dried at 60 °C for 24 hours and the dry weight was recorded to the nearest 0.01 g. Invertebrates were preserved in 10% formalin in 50% seawater and later identified to species level and counted.

Polychaetes were sampled separately from other benthic invertebrates because of their delicate nature and different sampling techniques. Three samples were taken using

sediment cores of 5 cm diameter and 5cm depth ($\sim 98.1 \text{ cm}^3$) in proximity to each invertebrate quadrat. Contents of the three cores were pooled and placed in a labelled plastic bag. In the laboratory sediments were gently wet sieved using a $500 \mu\text{m}$ mesh and the organisms, mainly polychaetes, were preserved in vials containing 70% ethanol in 50% seawater. These were later identified, only when heads were present, to species level and counted.

Physico-chemical variables

Salinity

Sediment pore water was collected using 20 ml air tight containers from holes formed by removing benthic fauna. These samples were placed in a 4-5 °C fridge overnight to allow any sediment to settle and to minimise temperature variation. Salinity was then measured (in parts per thousand, ppt) using a handheld YSI 30 salinity, conductivity and temperature system.

Dissolved oxygen

Dissolved oxygen (mg/l^{-1}) in sediment pore water was measured at sites with a handheld YSI 95 dissolved oxygen and temperature system. This was measured in pools formed at each site/plot by removing the four benthic samples. Measurements were taken at the end of sampling to avoid an inaccurate reading resulting from seepage. Water that had seeped into a hole during a trial had a consistent level of dissolved oxygen for 45 minutes.

Temperature

Temperature (°C) of sediment pore water was measured in pools formed by removal of benthic invertebrate samples using the YSI 95 meter. The reading was taken after enough water covered the sensor.

Sediment characteristics

Sediment corers were inserted to a depth of 5 cm after careful removal of surface macroalgae; this was to ensure that differences in organic matter including fine particulate organic matter (FPOM) and coarse particulate organic matter (CPOM) were not due to above surface macroalgae.

Pore water and percentage of organic matter

One sediment sample was taken using a 5cm diameter, 5cm depth (volume $\sim 98.12 \text{ cm}^3$) corer in proximity to each of the four invertebrate quadrats. Each sample was placed in a labelled airtight plastic container. In the laboratory wet weight was recorded to the nearest 0.01g using analytical scales. After oven drying for 3 days at 60 °C, dry weight was recorded to the nearest 0.01g. Percentage of pore water was calculated as: (wet weight - dry weight)/ wet weight x 100. Loss of volatile solids was used to measure the percentage of organic matter. Fifty grams of each dried sediment sample was gently ground to break up aggregates and ashed in a muffle oven at 500 °C for 5 hours to remove volatile solids from the sediment. Post ash weight was recorded to the nearest 0.01g. Percentage of volatile solids was calculated as: (pre-ashed weight – post-ashed weight)/ pre-ashed weight x 100.

Sediment particle size

Sediment samples were analysed for particle size composition using a Saturn DigiSizer 5200TM. Ten grams of each post-ashed sample were gently sieved to break up conglomerations, and immersed for one day in a 40 ml beaker containing 6% hydrogen peroxide in distilled water to dissolve remaining ash. This was periodically stirred to suspend the sample. The sample was then left to settle overnight. The following day supernatant liquid was removed by pipette, and the treatment was repeated. The sample was then rinsed twice with distilled water following the procedure above. Five ml of calgon was added (50 g/l) to the last rinse to prevent flocculation of fine particles. Samples were then placed in a fume cupboard until distilled water had evaporated and sediment was in a wet mud consistency. A sub-sample of this sediment was then analysed using the Saturn DigiSizer 5200TM. Each sample was analysed three times. Between each analysis the sample was internally redistributed by the machine. This accounted for particles that may have been obscured from the laser beam. Resulting data (particle diameter (μm) vs. volume frequency percent, mean of three tests) were grouped according to the Wentworth Scale (Wentworth 1922) (Table 2.1) using Excel spreadsheets. The mean of four samples per plot/site and standard error (S.D./ \sqrt{N}) was calculated. Analysis techniques and technical specifications for the Saturn DigiSizer 5200TM can be found by visiting:

www.pss.aus.net/products/micromeritics/equip_particle_size/5200/5200.html.

Table 2.1: Wentworth size class and grade limits, and grade limits measured by the DigiSizer. Acronyms used in this study are in parenthesis.

| Wentworth Size Class | Grade Limits | | DigiSizer Grade Limits | |
|-------------------------|--------------|----|------------------------|----|
| Boulder | >256 | mm | | |
| Cobble | 256 - 64 | mm | | |
| Pebble | 64 - 4 | mm | | |
| Gravel | 4 - 2 | mm | | |
| Very coarse sand (VCSA) | 2 - 1 | mm | 2-1 | mm |
| Coarse sand (CSA) | 1 - 0.5 | mm | 0.945 - 0.501 | mm |
| Medium sand (MSA) | 500 - 250 | µm | 473.87 - 251.57 | µm |
| Fine sand (FSA) | 250 - 125 | µm | 237.49 - 126.08 | µm |
| Very fine sand (VFSA) | 125 - 63 | µm | 119.03 - 63.19 | µm |
| Coarse silt (CSI) | 63 - 31 | µm | 59.65 - 31.67 | µm |
| Medium silt (MSI) | 31 - 15.6 | µm | 29.89 - 15.87 | µm |
| Fine silt (FSI) | 15.6 - 7.8 | µm | 14.98 - 7.95 | µm |
| Very fine Silt (VFSl) | 7.8 - 3.9 | µm | 7.51 - 3.98 | µm |
| Clay (C) | <3.9 | µm | <3.76 | µm |

Condition Index on the cockle Austrovenus stutchburyi

On collection day, the process for calculating the C.I. was begun. This was to prevent inaccurate indices being calculated because of deterioration in health of individuals from storage over time. One cockle was selected haphazardly from each of the four benthic samples from all plots/sites. Shells were cleaned to remove polychaete tubes and measured across the widest part using electronic callipers to the nearest 0.01 mm. Following this, shells were opened and washed to remove sediment from within the mantle. Excess water from washing was absorbed using strips of WettexTM absorbent cloth. Total weight of intact individuals was recorded using analytical scales to the nearest 0.001g. Tissue was excised onto a dry strip of WettexTM to absorb remaining excess water. Following this tissue was placed on a piece of pre-weighed aluminium foil and wet weight recorded to the nearest 0.001 g. Shells were dried and weighed as above. Finally, tissue and shells were dried at 60 °C for three days after which both dry weights were recorded to the nearest 0.001 g. The C.I. was calculated as: (dry tissue weight/dry shell weight) x 100.

2.8 Results

Quadrat and sample size

Species richness was found to be highest in the 0.0625 m² quadrat (n=20) (Table 2.2). Numbers of all invertebrates (m²) were consistently higher in the 0.0625 m² quadrat. The most abundant species were *A. crenata* and *A. stutchburyi* (Table 2.2). Variance/mean ratio was low for all animals found in the 0.0625 m² quadrats with exception of *Zeacumantus subcarinatus* (Table 2.2). This shows its distribution was highly clumped compared to other species. Variance/mean ratio for *Diloma subrostrata* and *Cominella glandiformis* were less than 1, therefore their spatial distribution was almost uniform. This was consistent when comparing distribution of *C. glandiformis* from 0.0225 m² quadrats. *Helice crassa* and *Notoacmea helmsi* were shown to have an almost uniform distribution when sampled with 0.0225 m² quadrats with a variance/mean ratio of less than 1. The same species was shown to have a clumped distribution when using the 0.0625 m² quadrat (Table 2.2.).

Table 2.2: Comparison between the 0.0225 m² and 0.0625 m² quadrat sizes in the mean (\pm S.D.), variance/mean ratio, number of species per m² and total species richness.

| Benthic invertebrates | 0.0225 m ² | | | 0.0625 m ² | | |
|---------------------------------|---|----------------|------------------------|---|----------------|------------------------|
| | Mean (\pm S.D.) 0.0225 m ² | Var/mean ratio | Mean (m ²) | Mean (\pm S.D.) 0.0625 m ² | Var/mean ratio | Mean (m ²) |
| <i>Helice crassa</i> | 0.25(0.44) | 0.79 | 11.11 | 2.05(1.61) | 1.26 | 32.8 |
| <i>Cominella glandiformis</i> | 0.10(0.31) | 0.95 | 4.44 | 0.65(0.75) | 0.85 | 10.4 |
| <i>Amphibola crenata</i> | 1.65(1.81) | 2.00 | 73.33 | 6.70(3.06) | 1.40 | 107.2 |
| <i>Zeacumantus subcarinatus</i> | 0.10(0.45) | 2.00 | 4.44 | 1.00(2.51) | 6.32 | 16 |
| <i>Zeacumantus lutulentus</i> | 0.20(0.52) | 1.37 | 8.88 | 0.95(1.00) | 1.05 | 15.2 |
| <i>Micrelenchus tenebrosus</i> | 0 | 0.00 | 0 | 0.05(0.22) | 1.00 | 0.8 |
| <i>Diloma subrostrata</i> | 0 | 0.00 | 0 | 0.10(0.31) | 0.95 | 1.6 |
| <i>Potamopyrgus estuarinus</i> | 0 | 0.00 | 0 | 0.45(1.00) | 2.22 | 7.2 |
| <i>Notoacmea helmsi</i> | 0.15(0.37) | 0.89 | 6.66 | 1.20(1.32) | 1.46 | 19.2 |
| <i>Austrovenus stutchburyi</i> | 0.95(1.19) | 1.49 | 42.22 | 5.00(3.20) | 2.04 | 80 |
| <i>Macomona liliana</i> | 0 | 0.00 | 0 | 0.50(0.95) | 1.79 | 8 |
| <i>Anthopleura aureoradiata</i> | 0.10(0.45) | 2.00 | 4 | 0.65(0.81) | 1.02 | 10.4 |
| Total species | 8 | | | 12 | | |

Precision of sampling increased with number of quadrats (Fig. 2.4). Confidence interval for 0.0625 m² quadrats was lower than 0.0225 m² quadrats. Therefore precision of sampling was higher for 0.0625 m² quadrats. Greater precision at the 20% confidence interval was also reached much earlier in 0.0625 m² quadrats compared to 0.0225 m² quadrats (Fig. 2.4). Maximum species richness was reached in fewer samples using 0.0625 m² quadrats compared to 0.0225 m² quadrats (Fig. 2.5).

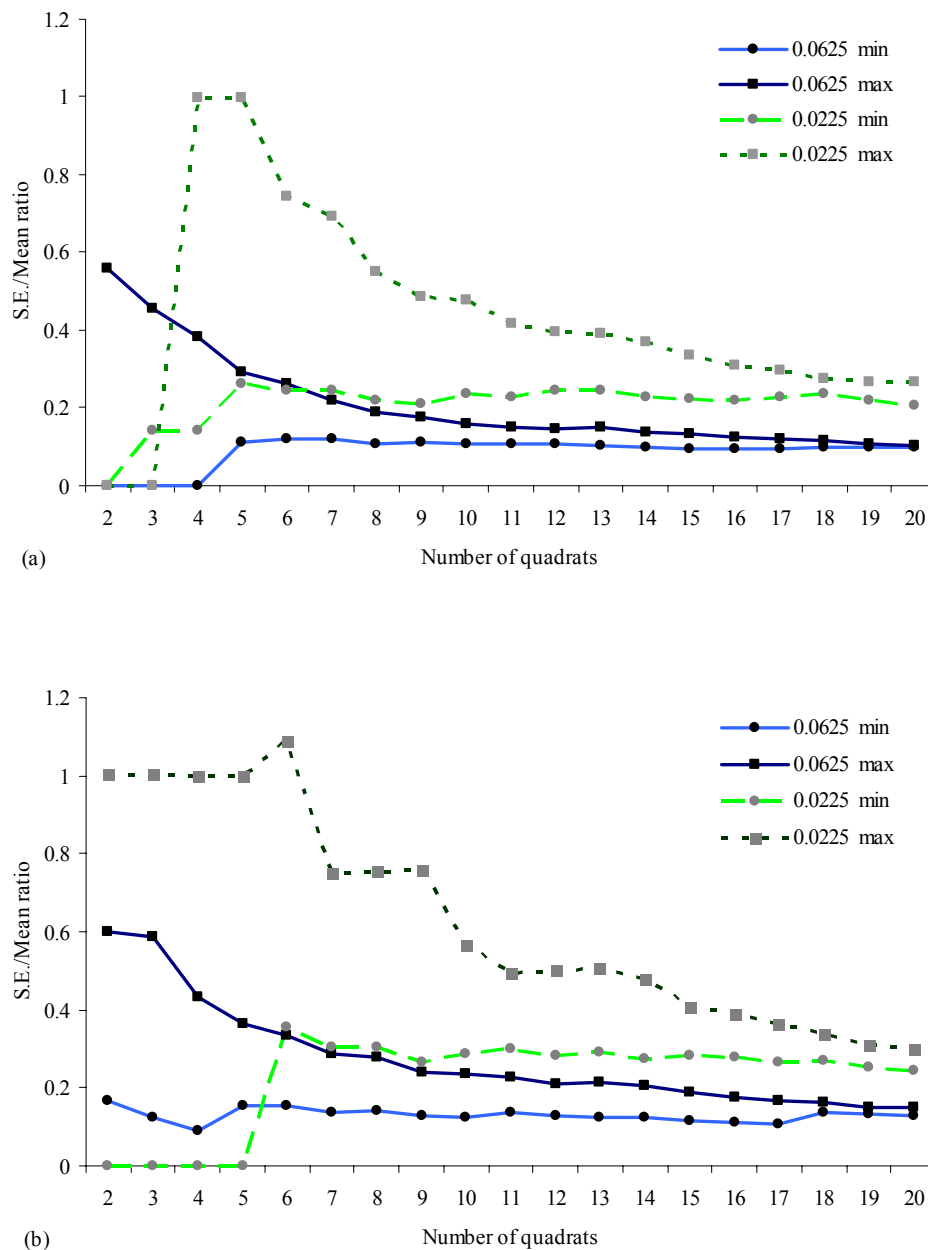


Figure 2.4: Comparison between the 0.0625 m² and 0.0225 m² quadrats in 20% confidence intervals, minimum (Min), and maximum (Max) of precision (standard error/mean ratio) with increasing quadrat number for (a) *Amphibola crenata* and (b) *Austrovenus stutchburyi*.

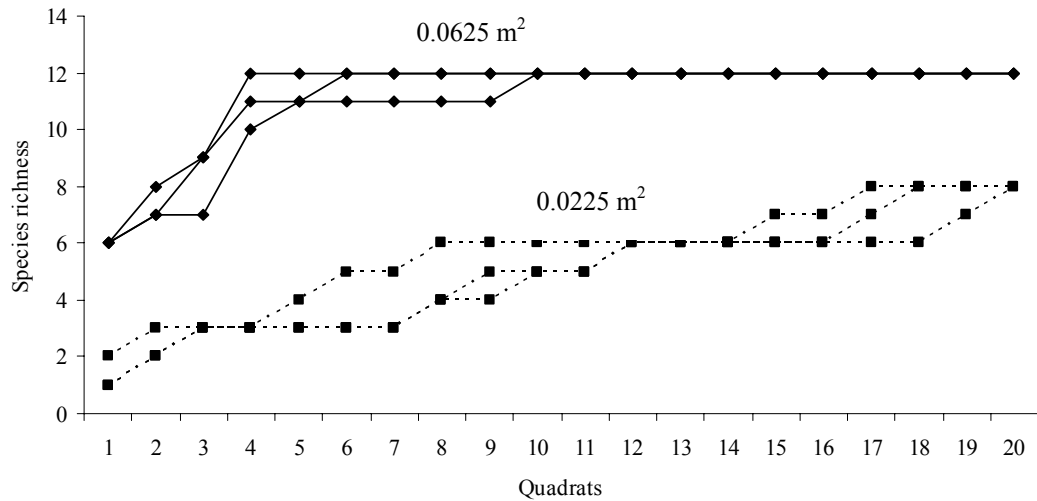


Figure 2.5: A comparison of the cumulative species richness with increasing quadrat numbers between the 0.0625 m² and 0.0225 m² quadrats. The order of quadrats was rearranged 3 times at random for each size.

Comparison of sediment analysis methods

There was a significant difference between DigiSizer and manual methods of sediment analysis when comparing results from FSA sediment class (ANOVA, $P_{(df=1, F=539.07)} < 0.001$; Fig.2.6). There were no significant differences between the two methods when comparing whole samples from S6A, S6D and S8D (ANOVA, $P_{(df=1, F=0.26)} = 0.62$; ANOVA, $P_{(df=1, F=0.55)} = 0.47$; ANOVA, $P_{(df=1, F=0.06)} = 0.81$ respectively; Fig.2.7).

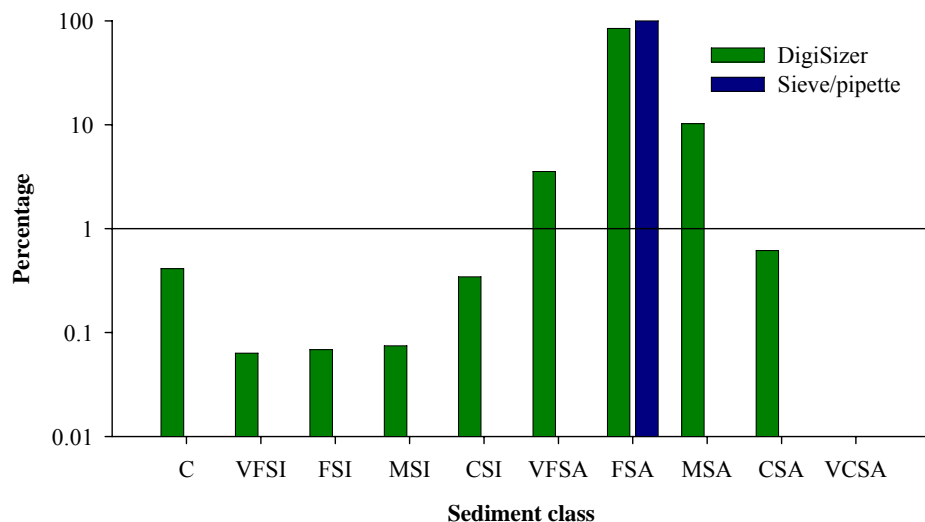


Fig 2.6: Sediment analysis by the DigiSizer of the FSA sediment class from the sieve/pipette method. C = clay, VFSI = very fine silt, FSI = fine silt, MSI = medium silt, CSI = coarse silt, VFSA = very fine sand, FSA = fine sand, MSA = medium sand, CSA = coarse sand, VCSA = very coarse sand.

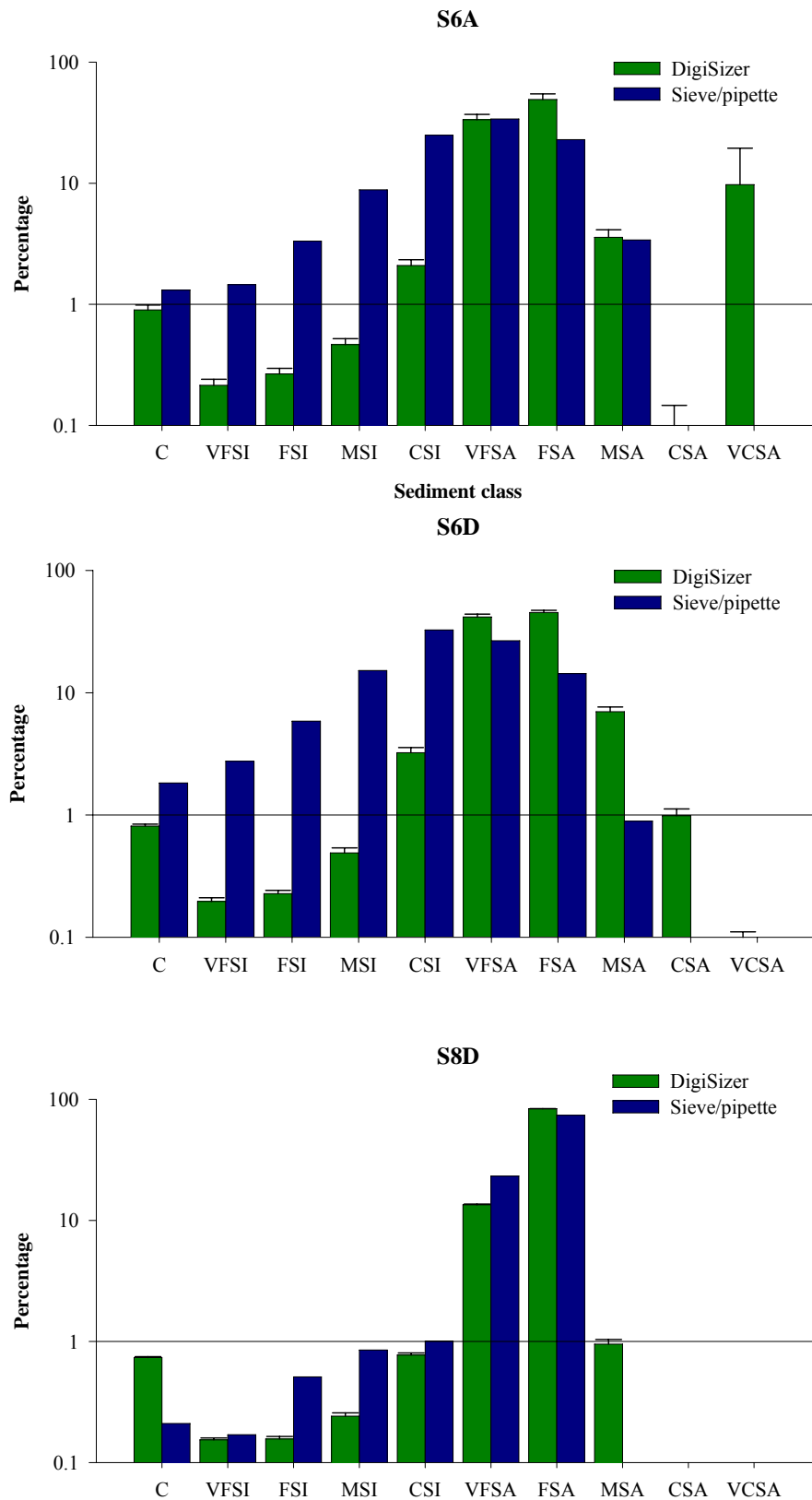


Figure 2.7: The mean (\pm S.E.) percentage sediment class as analysed by the DigiSizer and the percentage of sediment class as processed by manual method. C = clay, VFSl = very fine silt, FSl = fine silt, MSi = medium silt, CSi = coarse silt, VFSA = very fine sand, FSA = fine sand, MSA = medium sand, CSA = coarse sand, VCSA = very coarse sand. No standard error was available for the samples processed by the traditional method as only one complete sample per comparison was available.

*Removal of *Ulva lactuca* mats*

Removal of *Ulva lactuca* took a little over 2 minutes when using the mechanical broom. Removal by raking took 17 minutes. It was noted that numerous shreds of *U. lactuca* were not removed by rake. The plot cleared by mechanical broom was virtually devoid of *U. lactuca* with only a few small shreds remaining. There was also evidence of greater disturbance to the plot treated by rake. This included trampling and bivalves that had been pulled out from the sediment. Crabs were seen to have been badly injured by the rakes prongs; the carapace had been cracked or pierced. As the operator of the rake walked backwards, other epifauna species were seen to be trampled into the sediment. The plot treated by mechanical broom was relatively undisturbed with exception of uppermost surface sediments being swept away. When measured there was a 0.20 cm difference between levels of treated and untreated areas. It was observed that fewer epifaunal species had been trampled into the sediment and no bivalves were seen to be pulled out of the sediment. Also, there were fewer injured crabs resulting from this removal technique.

Preliminary study

Sediment Characteristics

At site MB the mean fraction of silt/clay was 72.1% and ranged from 60.9% to 79.3% (Fig 2.8). There were significant variations in percentage of sediment classes between plots at site MB (ANOVA, $P_{(df=11, f=9.996)} < 0.001$; Table 2.3).

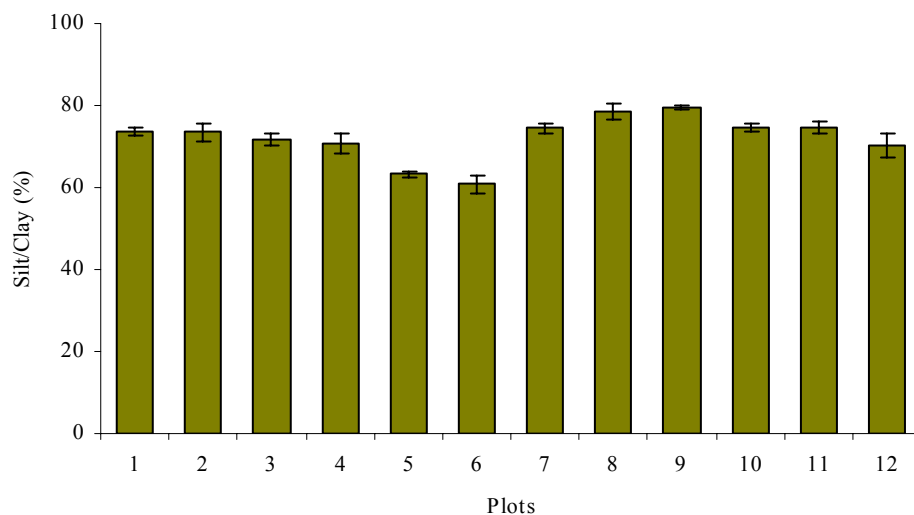


Figure 2.8: The mean (\pm S.E.) percentage of silt/clay in all plots at site McCormacks Bay.

Table 2.3: ANOVA results for the variation between plots in the mean classes of sediment (square-root-transformed and normalised) at McCormacks Bay. Size classes are as defined in Table 2.1.

ANOVA

| Source of Variation | Df | Sum Sq | Mean Sq | F-value | P-value |
|---------------------|----|--------|---------|---------|---------|
| Between Groups | 11 | 35.408 | 3.219 | 9.996 | <0.001 |
| Within Groups | 36 | 11.592 | 0.322 | | |
| Total | 47 | 47 | | | |

There was low variation between plots in dissolved oxygen of sediments' pore water, temperature, and salinity (Fig. 2.9). There were significant variations between plots in the temperature, volatile solids, pore water, and *U. lactuca* dry weight biomass (Table 2.4). There were no significant variations between plots in dissolved oxygen and salinity (Table 2.4).

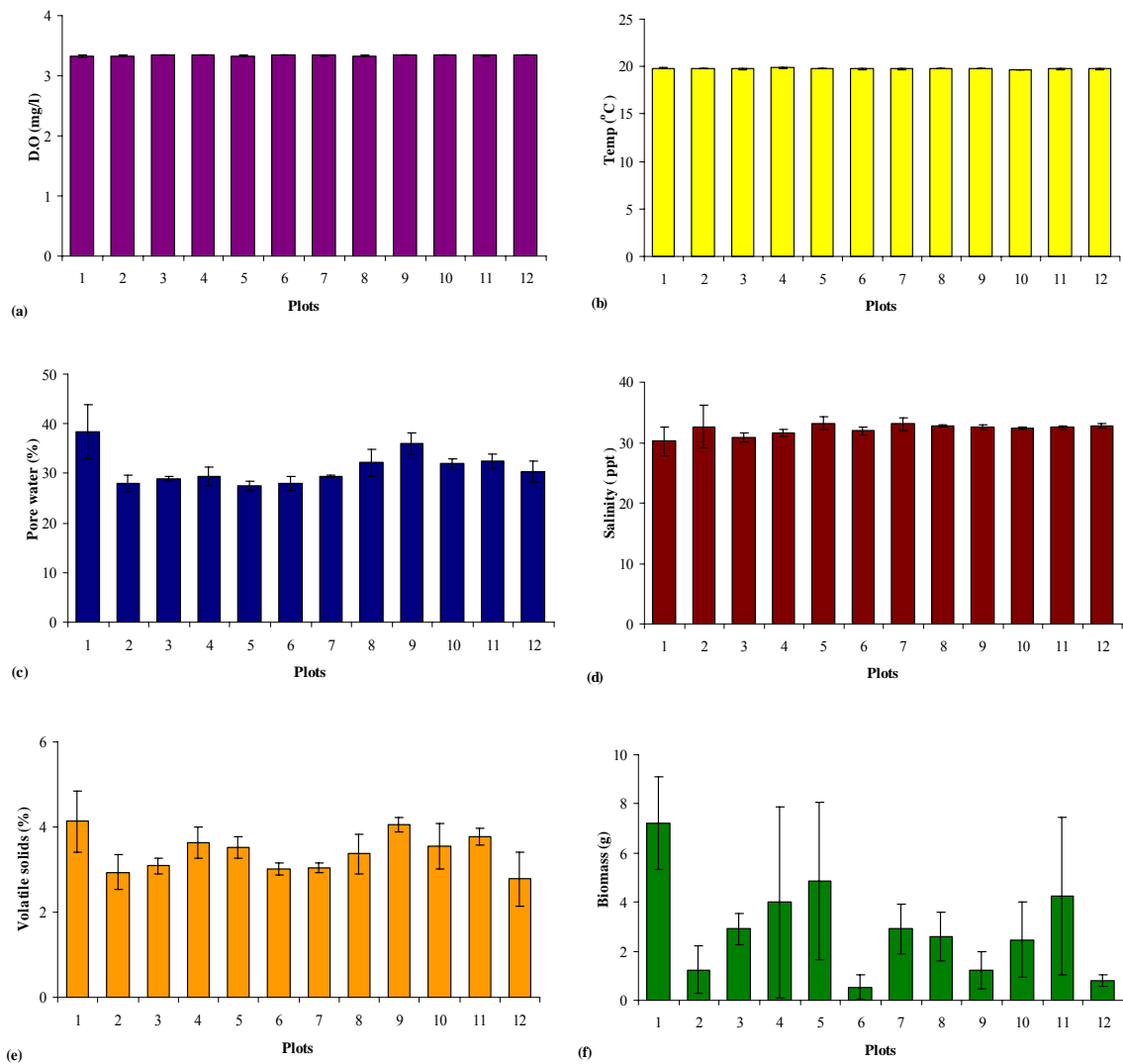


Figure 2.9: Mean (±S.D.) physico-chemical variables (a) dissolved oxygen (mg l^{-1}), (b) temperature ($^{\circ}\text{C}$) (c) pore water (%), (d) salinity (ppt), (e) volatile solids (%), and (f) dry weight biomass of *Ulva lactuca* from each of the 12 plots at McCormacks Bay.

Table 2.4: Summary ANOVA results for the variation between plots in the physico-chemical variables: dissolved oxygen (mg/l⁻¹), temperature (°C), pore water (%), salinity (ppt), volatile solids (%), and *Ulva lactuca* dry weight biomass (g) McCormacks Bay. Data were (square-root-transformed and normalised when appropriate).

| ANOVA | | |
|--|--------|---------|
| Variable | F | P |
| Dissolved oxygen (mg/l ⁻¹) | 1.450 | = 0.194 |
| Temperature (°C) | 4.169 | < 0.001 |
| Salinity (ppt) | 1.769 | = 0.097 |
| Volatile solids (%) | 4.754 | < 0.001 |
| Pore Water (%) | 10.038 | < 0.001 |
| <i>Ulva lactuca</i> dry wgt. (g) | 10.967 | < 0.001 |

The correlations between physico-chemical variables and *U. lactuca* dry weight biomass showed that pore water was positively correlated with *U. lactuca* biomass (Table 2.5). There were also positive correlations between pore water and volatile solids, pore water and sediments, and volatile solids and *U. lactuca* biomass. The only negative correlation was between *U. lactuca* biomass and salinity. Volatile solids were positively correlated with temperature (Table 2.5).

Table 2.5: Significant correlations between physico-chemical variables temperature (°C), pore water (%), salinity (ppt), volatile solids (%), and *Ulva lactuca* biomass dry weight (g) at McCormacks Bay.

| Physico-chemical variables | Correlations (r ²) |
|---|--------------------------------|
| Volatile solids and temperature | 0.365* |
| Volatile solids and <i>Ulva</i> spp. dry weight biomass | 0.323* |
| Pore water and volatile solids | 0.647** |
| Pore water and <i>Ulva</i> spp. dry weight biomass | 0.489 ** |
| Pore water and sediment | 0.518** |
| <i>Ulva lactuca</i> dry weight biomass and salinity | -0.388** |

* Correlation is significant at (α) = 0.05 (2-tailed)

**Correlation is significant at (α) = 0.01 (2-tailed)

Benthic invertebrates

Eleven species from 4 orders contributed to the 1465 individuals recorded from preliminary sampling at site MB (Table 2.6). Most abundant species were *Microtenella* *tenebrosus* and *Arthritica* *bifurca*. Least abundant species was *Diloma* *nigerrima* (Table 2.6).

All species had a clumped distribution with *A. bifurca* being most clumped. The only positive correlations between physico-chemical variables and *Ulva* spp. biomass were between *Notoacmea helmsi*, sediment organic matter and *U. lactuca* biomass (Table 2.7). *Austrovenus stutchburyi* and *N. helmsi* were negatively correlated with salinity; species numbers decreased as salinity increased (Table 2.7). Negative correlations were shown between *Helice crassa*, *M. tenebrosus*, *Zeacumantus subcarinatus* and dissolved oxygen in sediment pore water (Table 2.7). *Cominella glandiformis* and *H. crassa* were the only species positively correlated with percentage of silt/clay ($r^2 = 0.386^{**}$ and $r^2 = 0.314^*$ respectively).

Table 2.6: The mean abundance (\pm S.D.) of benthic species in 0.0625 m² (excluding polychaetes) and their total numbers from all plots at McCormacks Bay.

| Group | Species | \bar{X} (0.0625m ²) | Variance/mean ratio | Total individuals |
|------------|---------------------------------|-----------------------------------|---------------------|-------------------|
| Decapoda | <i>Helice crassa</i> | 2.02(2.07) | 2.12 | 97 |
| Gastropoda | <i>Cominella glandiformis</i> | 0.94(1.10) | 1.29 | 45 |
| Gastropoda | <i>Amphibola crenata</i> | 2.71(2.43) | 2.18 | 130 |
| Gastropoda | <i>Micrelenchus tenebrosus</i> | 6.85(5.92) | 5.11 | 329 |
| Gastropoda | <i>Diloma nigerrima</i> | 0.19(0.57) | 1.74 | 9 |
| Gastropoda | <i>Zeacumantus subcarinatus</i> | 3.23(2.12) | 1.39 | 155 |
| Gastropoda | <i>Zeacumantus lutulentus</i> | 2.94(2.24) | 1.72 | 141 |
| Gastropoda | <i>Notoacmea helmsi</i> | 1.02(1.12) | 1.23 | 49 |
| Bivalvia | <i>Austrovenus stutchburyi</i> | 3.42(3.39) | 3.37 | 164 |
| Bivalvia | <i>Arthritica bifurca</i> | 4.67(8.43) | 15.23 | 224 |
| Anthozoa | <i>Anthopleura aureoradiata</i> | 2.54(2.11) | 1.76 | 122 |

Table 2.7: Significant correlations between benthic invertebrates and physico-chemical variables (dissolved oxygen (D.O.), temperature (°C), pore water (%), salinity (ppt), volatile solids (%)) and *Ulva lactuca* biomass dry weight (g) at site McCormacks Bay. Species are listed by group (Decapoda, Gastropoda, Bivalvia, and Anthozoa).

| Group | Species | D.O. (mg/l ⁻¹) | Temp (°C) | Pore water (%) | Salinity (ppt) | Volatile solids (%) | <i>Ulva</i> spp. biomass (g) |
|------------|---------------------------------|-------------------------------|--------------|----------------------|-------------------|---------------------------|---------------------------------------|
| Decapoda | <i>Helice crassa</i> | -0.363* | - | - | - | - | - |
| Gastropoda | <i>Cominella glandiformis</i> | - | - | - | - | - | - |
| Gastropoda | <i>Amphibola crenata</i> | - | - | - | - | - | - |
| Gastropoda | <i>Micrelenchus tenebrosus</i> | -0.289* | - | - | - | - | - |
| Gastropoda | <i>Diloma nigerrima</i> | - | - | - | - | - | - |
| Gastropoda | <i>Zeacumantus subcarinatus</i> | - | - | - | - | - | - |
| Gastropoda | <i>Zeacumantus lutulentus</i> | -0.292* | - | - | - | - | - |
| Gastropoda | <i>Notoacmea helmsi</i> | - | - | - | -0.323* | 0.310* | 0.391** |
| Bivalvia | <i>Austrovenus stutchburyi</i> | - | - | - | -0.408** | - | - |
| Bivalvia | <i>Arthritica bifurca</i> | - | - | - | - | - | - |
| Anthozoa | <i>Anthopleura aureoradiata</i> | - | - | - | - | - | - |

* Correlation is significant at (α) = 0.05 (2-tailed)

**Correlation is significant at (α) = 0.01 (2-tailed)

2.9 Discussion

The majority of benthic invertebrate species found during quadrat and sample assessment had a clumped distribution (Table 2.2). Clumping of species increases probability of under or overestimating species abundance and assemblage at a location; finding or missing clumped individuals with the quadrat would affect precision of sampling. Greater precision, and a fewer number of quadrats needed to obtain precise representations of species assemblages and their abundances, were shown to be achieved using 0.0625 m² quadrats (Fig. 2.4 and 2.5). This occurred when using four or more quadrats. Based on this, four 0.0625 m² quadrats were used in each plot during the preliminary study at site MB.

There were advantages of using the Saturn DigiSizer as opposed to manual sieve and pipette methods for analysis of sediment particle composition. The greatest advantage was time taken to process each complete sample; DigiSizer took 22 minutes to process one sample. In this time the sample was redistributed three times (during this time the operator could concentrate on other tasks such as data organisation). This allowed measurement of a standard deviation for three runs. It took approximately one and a half hours to run four separate replicates from one plot. This resulted in low standard error and greater precision (Fig. 2.7). The manual pipette method took two hours and three minutes to complete one sample and only provided data for the silt/clay fraction. The method relied on the operator taking samples with a pipette at precise times and levels from a measuring cylinder. Accidental knocking of the cylinder or the pipette failing to draw properly results in an aborted procedure. Further time was taken for evaporation and drying of samples before weighing could commence. During this time accidental loss of particles could occur from handling.

Accuracy of the DigiSizer was assessed by running a single particle size (FSA) that was also processed by manual methods; DigiSizer analysis showed five particle sizes present in the sample which were not detected by the manual method (Fig. 2.6). This showed that a greater accuracy was achieved with this method. DigiSizer was selected as the preferred analysis technique because high numbers of samples required processing and the effective equivalence of the two methods. This would allow for a confident comparison between results of the current study using DigiSizer and previous and future studies using the traditional pipette method.

The mechanical broom was chosen as preferred tool for removing *U. lactuca*. This was based on potential cost, time taken and observed level of impact to estuarine biota and the environment. Although there would be cost associated with initial purchase, ongoing running and maintenance of the mechanical broom, these would be offset by a decrease in labour and impact on the environment. With the mechanical broom, personnel would spend less time on estuarine mudflats, and there would be less trampling, as shown by observed impacts when comparing the two treatments (refer to section 2.8, page 30).

The preliminary study at site McCormacks Bay was completed successfully in time between tides. It took four people approximately three hours to collect and transport samples from the site. Each person was assigned three plots and equipped with pre-prepared sets of sampling equipment. Sampling was difficult because of sinking in soft sediments high in silt/clay (Fig. 2.8); however, care was taken where possible to avoid disturbing experimental plots. Sampling techniques proved reliable with no significant variation in standard deviation of physico-chemical samples from each plot. The exception to this was in *U. lactuca* dry weight biomass (Fig. 2.9). This was expected because cover was thicker in some samples. Standard error was low in all plots for sediment particle composition. This raised confidence in consistency of analysis by DigiSizer. Benthic invertebrates were found to have a clumped distribution (Table 2.6) as were invertebrates collected during assessment near Raupo Bay (Table 2.2). Laboratory work was initially intensive and priority was given to perishable samples such as invertebrates and macroalgae. It was necessary to preserve invertebrates after overnight storage in a 5°C fridge. Macroalgae were processed on the day of sampling.

The preliminary study allowed practise of sampling times and methods to be determined. Consideration had to be given to order and volume of samples requiring processing. This was based on the preliminary study. It was concluded that four benthic invertebrate samples and sediment cores per plot was a practical number to sample in the field and process back at the laboratory. Four samples were also found to give a precise representation of assemblages and their abundance based on results of quadrat and sample assessments. In this instance, consideration was given to length of time involved for identifying and counting species without overburdening thereby compromising the accuracy of the sample examined. The processing and calculation of cockle condition indices was also to be included for the removal experiment. Therefore, it was decided to separate the times of sampling between sites by one month to allow for processing of samples.

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(Cardoso *et al.* 2004a)

Chapter 3

General survey

3.1 Introduction

Estuarine systems have a diversity of niches that are inhabited by a wide range of benthic invertebrates from groups including decapods, gastropods, bivalves and polychaetes (Schubel 1971; McLusky 1981; Robertson *et al.* 2002; Hume 2003). Distribution, abundance and diversity of these invertebrates is limited by tolerance to gradients in physical variables including salinity, temperature and sediment particle composition (Knox and Kilner 1973; Yates *et al.* 1993; Blanchard and Bourget 1999; Pihl *et al.* 1999; Bolam and Fernandes 2002; Edgar and Barrett 2002). The majority of benthic invertebrates, such as the bivalve *Austrovenus stutchburyi* and the crab *Hemigrapsus crenulatus* are distributed within mid and low tide zones because of an inability to tolerate prolonged exposure to the elements (Jones 1976; Bonsdorff *et al.* 1995; Marsden 2004). Other species, such as the tunnelling mud crab *Helice crassa* and mudflat snail *Amphibola crenata*, are able to tolerate wide ranges in physical conditions and long periods of exposure (Jolly 1971; Jones 1976; Griffin and Thomson 1992). Species such as these are widely distributed.

Benthic invertebrates are affected by natural and anthropogenic processes that occur over a variety of temporal and spatial scales (Hewitt *et al.* 1998; Blanchard and Bourget 1999; Edgar and Barrett 2002; Hewitt *et al.* 2002; Cardoso *et al.* 2004b). Processes such as silt deposition from riverine sources and effluent discharge reduce water depth, clarity and quality. These contribute to shifts from coarser sand particles to finer silt/clay particles on estuarine mudflats (Schubel 1971; Raffaelli *et al.* 1999). This can cause a reduction in Redox Discontinuity Layer (RDL) (transition zone between the aerobic (oxygenated) sediments and the anerobic (deoxygenated) sediments) which is also correlated with formation of macroalgal mats and hydrogen sulphide (Theede *et al.* 1969). The result is declining numbers of species (Thiel and Watling 1998; Bolam *et al.* 2000; Bressington 2003). Infaunal species such as the bivalves *A. stutchburyi* and *Macomona liliana* are rarely found beneath the RDL whereas species such as *Capitella capitata*, a polychaete, are more resistant to oxygen-deficiency and increase in numbers (Theede *et al.* 1969; Norkko and Bonsdorff 1996a; Raffaelli 2000).

Long-term ecological research, such as many studies carried out in the Ythan Estuary, Aberdeenshire, Scotland (Balls *et al.* 1995; Gillibrand and Balls 1998; Raffaelli 1999; Raffaelli *et al.* 1999; Raffaelli 2000) and the Avon-Heathcote Estuary, Canterbury, New Zealand (Estcourt 1967; Knox and Kilner 1973; Steffensen 1974; Steffensen 1976; Stephenson 1981; Marsden and Pilkington 1995; Bressington 2003; Maclaren 2005) are appropriate for detecting changes in community assemblages of these systems. Such research contributes to an increased understanding of potentially important ecological changes at various temporal and spatial scales that affect valuable ecosystems such as estuaries (Raffaelli 1992; Edgar and Barrett 2002).

There are difficulties in making broad generalisations from results of studies such as these (Beck 1997; Menge *et al.* 1997). This is because detection of gradients is somewhat dependant on sample size and number taken at each location; increasing number and size of samples leads to greater heterogeneity in both assemblages and physical variables (Thrush 1991; Schneider 1994; Thrush *et al.* 2000). This can result in collapse of obvious patterns and processes to noise (Thrush *et al.* 1997). Difficulties then arise in mechanistic explanations of processes that drive variability in diversity and abundance within a particular system (Beck 1997). Sampling at regular intervals such as monthly, seasonally, or annually contributes to the detection and understanding of the processes that generate variability (Thrush *et al.* 2000). Therefore, there are benefits of comparative studies over time, as these are likely to reveal trends and broaden understanding of the local ecological relationships (Menge and Sutherland 1976; Beck 1997; Thrush *et al.* 1997).

In the present study, seven sites in the Avon-Heathcote Estuary were located at spatial, and sampled at temporal scales, in which gradients in sediment physico-chemical variables were likely to change because of the proximity and distance from the Estuary mouth thus influencing composition and diversity of assemblages. These sites were the Causeway (CW), Channel (CH), Raupo Bay (RB), Ebb Tide Street (ES), Penguin Street (PS), Tern Street (TS) and McCormacks Bay (MB) (Fig. 3.1). The objective was to determine seasonal patterns of variation in assemblages of benthic invertebrates and physico-chemical variables. These were examined together to determine which variables correlated best with patterns of community assemblages. In addition, comparisons were made between community assemblages and physico-chemical variables from Bressington's December survey in 2003 and the December survey from the current study. This was to determine the comparability between studies using different sample sizes and numbers.

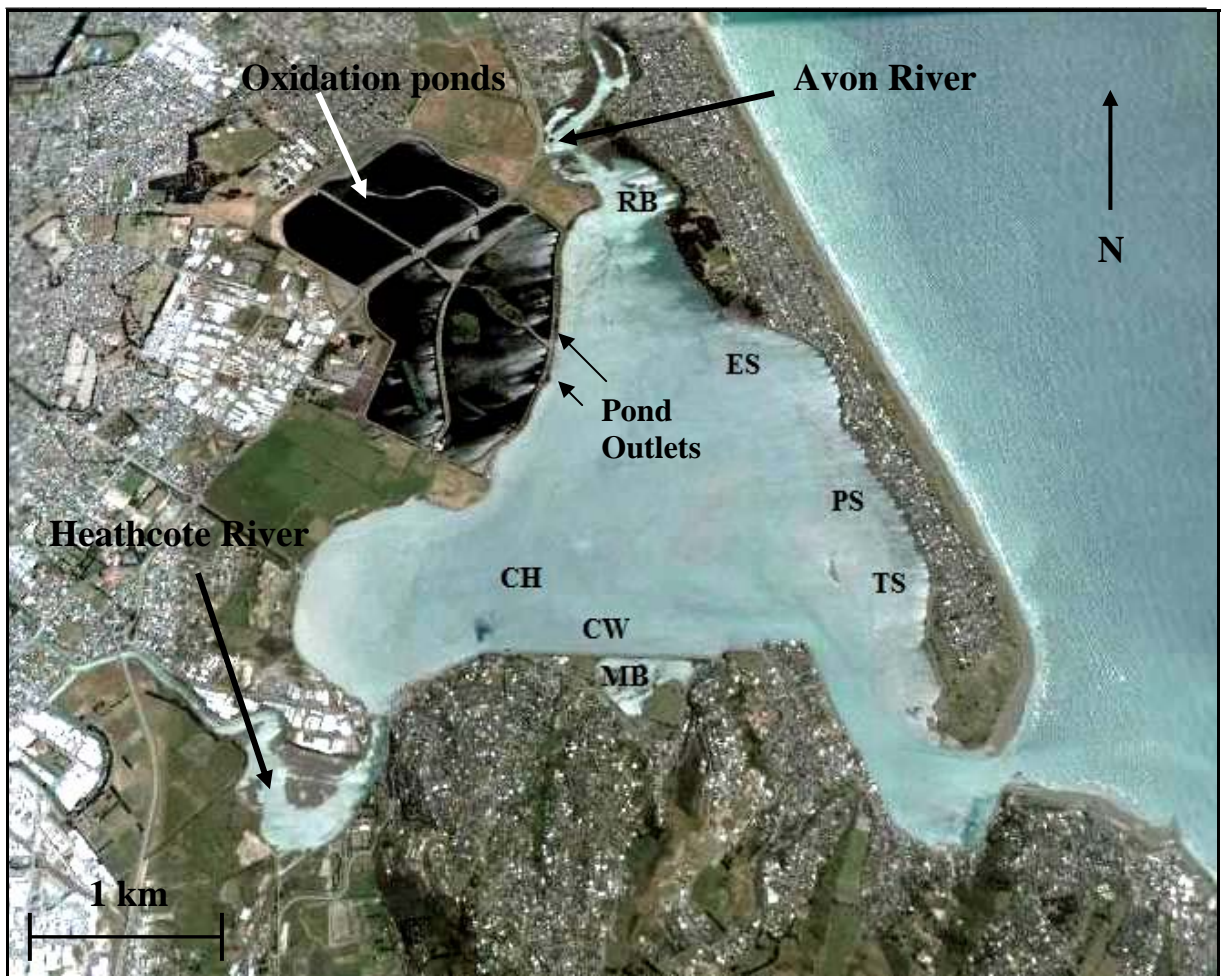


Figure 3.1: Satellite image of Avon-Heathcote Estuary showing locations of study sites, Causeway (CW), Channel (CH), Raupo Bay (RB), Ebb Tide Street (ES), Penguin Street (PS), Tern Street (TS) and McCormacks Bay (MB), entry of Avon and Heathcote Rivers, waste treatment works oxidation ponds, and pond outlets.

An additional field experiment was conducted during the general survey to test if substrate availability was a limiting factor for settlement and growth of *Ulva lactuca* L at sites where hard substrate and attached *U. lactuca* plants were rare.

Methods

Cleaned half cockle shells from *A. stutchburyi* were glued to tiles of marine plywood measuring 25 x 25 cm. Sediment from the sites where the tiles were to be placed was glued to the spaces between the cockle shells. In the mid-tide zone at each site (Channel, Raupo Bay, Ebb Tide Street, Penguin Street and Tern Street Fig. 3.1) eight tiles including two controls (tiles with sediment only) were distributed at random and fixed in place with metal pegs. Circular exclusion fences were fixed in place with metal pegs around three of the tiles with cockle shells and one without shells. Each fence had a turned down lip at 45 degrees facing away from the tiles. This was to prevent grazing gastropods from moving onto the tiles. The Channel site was selected as a control site because *U. lactuca* plants were observed to be common and attached to live *A. stutchburyi* and their empty shells.

3.2 Results

3.2.1 Benthic species

In total, 34 species were recorded during the study (Table 3.1). These were from four main groups that contributed to community diversity between sites: Decapoda, Gastropoda, Bivalvia and Polychaeta. Minor groups included Anthozoa, Polyplacophora and Cirripedia.

Table 3.1: Benthic species found at each site and their total numbers from all sites and sampling times in the Avon-Heathcote Estuary during the general survey. Presence is indicated by an X. Total dry weight (g) is given for benthic flora. Figure 1.3 shows the location of all sites.

| Groups | Species | CW | CH | RB | ES | PS | TS | MB | Total individuals |
|----------------|----------------------------------|----|----|----|----|----|----|----|-------------------|
| Decapoda | <i>Halicarcinus whitei</i> | X | X | X | X | X | X | | 68 |
| Decapoda | <i>Helice crassa</i> | X | X | X | X | X | X | X | 253 |
| Decapoda | <i>Hemigrapsus crenulatus</i> | X | X | | X | X | | | 37 |
| Decapoda | <i>Macrophthalmus hirtipes</i> | X | X | | X | X | X | | 121 |
| Gastropoda | <i>Micrelenchus tenebrosus</i> | X | X | | X | X | X | X | 1831 |
| Gastropoda | <i>Diloma subrostrata</i> | X | X | | | X | X | | 148 |
| Gastropoda | <i>Diloma nigerrima</i> | X | X | | X | X | X | X | 361 |
| Gastropoda | <i>Zeacumantus subcarinatus</i> | | | | | | | X | 130 |
| Gastropoda | <i>Zeacumantus lutulentus</i> | | | | | | | X | 70 |
| Gastropoda | <i>Potamopyrgus estuarinus</i> | X | | X | | | | | 189 |
| Gastropoda | <i>Amphibola crenata</i> | | | X | X | X | X | X | 1128 |
| Gastropoda | <i>Notoacmea helmsi</i> | X | X | | X | X | X | X | 745 |
| Gastropoda | <i>Xymene plebeius</i> | | X | | | | X | | 13 |
| Gastropoda | <i>Cominella glandiformis</i> | X | X | X | X | X | X | X | 163 |
| Bivalvia | <i>Austrovenus stutchburyi</i> | X | X | X | X | X | X | X | 2211 |
| Bivalvia | <i>Macomona liliana</i> | X | X | | X | X | X | | 834 |
| Bivalvia | <i>Arthritica bifurca</i> | X | X | X | X | X | X | X | 970 |
| Bivalvia | <i>Mytilus galloprovincialis</i> | X | X | | | | X | | 5 |
| Bivalvia | <i>Paphies australis</i> | X | X | X | X | X | X | | 113 |
| Polychaeta | <i>Scolecopides benhami</i> | X | X | X | X | X | X | X | 229 |
| Polychaeta | <i>Boccardia polybranchia</i> | X | X | X | | | | X | 266 |
| Polychaeta | <i>Pectinaria australis</i> | X | X | | | | | | 55 |
| Polychaeta | <i>Heteromastus filiformis</i> | X | X | | | X | X | | 186 |
| Polychaeta | <i>Nicon aestuariensis</i> | | | X | | | | X | 91 |
| Polychaeta | <i>Scoloplos cylindrifer</i> | X | X | | X | X | X | | 311 |
| Polychaeta | <i>Capitella sp.</i> | X | X | X | X | | | X | 124 |
| Sipuncula | Sipunculid worm | X | | X | X | | X | | 44 |
| Anthozoa | <i>Anthopleura aureoradiata</i> | X | X | | X | | X | X | 1010 |
| Amphipoda | <i>Melita awa</i> | | | | | | | X | 1 |
| Polyplacophora | <i>Amaurochiton glaucus</i> | | X | | | | | | 7 |
| Cirripedia | <i>Elminius modestus</i> | | X | | | | | | 39 |
| Chlorophyta | <i>Ulva lactuca</i> | X | X | X | X | | X | X | 93.33 (g) |
| Rhodophyta | <i>Gracilaria chilensis</i> | | | X | | | | | 8.39 (g) |
| Anthophyta | <i>Zostera novaezealandica</i> | | | | | X | X | | 119.66 (g) |

Total species richness at sites varied between 13 and 24, and mean species richness between 11 and 19 with the sites closest to the causeway, sites CH and CW, in the western side of the Estuary having the highest and site RB in the eastern side of the Estuary having the lowest number of species (Table 3.2). The seven most abundant species in order were: *Austrovenus stutchburyi*, *Microvelinus tenebrosus*, *Amphibola crenata*, *Anthopleura aureoradiata*, *Arthritica bifurca*, *Macomona liliana*, and *Notoacmea helmsi* (Table 3.1). The Berger-Parker Dominance Index showed these species, in their combinations at each site, accounted for over 50% of total individuals over all sampling dates (Table 3.2). The four rarest species overall were *Melita awa*, *Mytilus galloprovincialis*, *Amaurochiton glaucus* and *Xymene plebeius*, which were found at sites MB, CW, CH in the west, and site TS in the east side of the Estuary (Table 3.1). Total number of individuals of all species varied between sites with sites CW and CH having the most and site ES in the east side of the Estuary the least (Fig. 3.2).

Table 3.2: Total species richness and mean species richness at each site during the general survey. The Berger-Parker Dominance Index of the three main species from each site and all sampling times calculated as (total number of each species) / total individuals from that site.

| Site | Total species richness | Mean species richness | Berger-Parker Dominance Index | | |
|------|------------------------|-----------------------|--------------------------------|---------------------------------|---------------------------------|
| CW | 23 | 19 | <i>A. stutchburyi</i> 0.255 | <i>A. aureoradiata</i> 0.223 | <i>M. liliana</i> 0.139 |
| CH | 24 | 18 | <i>M. tenebrosus</i> 0.308 | <i>A. stutchburyi</i> 0.248 | <i>N. helmsi</i> 0.129 |
| RB | 13 | 11 | <i>A. bifurca</i> 0.358 | <i>P. estuarinus</i> 0.174 | <i>A. crenata</i> 0.153 |
| ES | 18 | 13.75 | <i>M. liliana</i> 0.202 | <i>A. crenata</i> 0.186 | <i>A. stutchburyi</i> 0.173 |
| PS | 17 | 14 | <i>A. crenata</i> 0.291 | <i>A. stutchburyi</i> 0.178 | <i>A. bifurca</i> 0.128 |
| TS | 20 | 16 | <i>M. tenebrosus</i> 0.271 | <i>A. crenata</i> 0.176 | <i>A. stutchburyi</i> 0.143 |
| MB | 16 | 13.75 | <i>A. bifurca</i> 0.177 | <i>A. crenata</i> 0.172 | <i>Z. subcarinatus</i> 0.135 |

At all sites, greater than 66% of individuals were a combination of gastropods and bivalves (Fig. 3.3). Sites PS and TS had equal highest at ~84%. Bivalves dominated sites CW and ES amounting to ~43% of the total individuals, while at site MB gastropods contributed to ~47% of the total individuals. The highest percentage of gastropods was at site TS where they contributed to ~60% of total individuals. Sites RB and MB had the highest proportions of polychaetes. Other groups, including anthozoans, were present at sites CW, CH, ES, and MB

with site CW having the highest proportion. Proportions of decapods were greatest at sites TS and PS and least at site CH (Fig. 3.3).

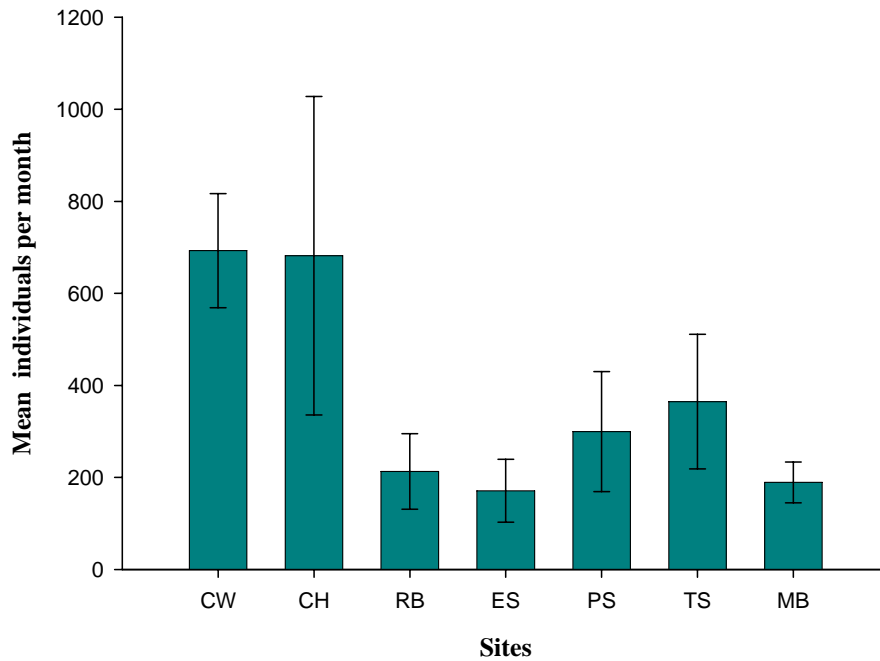


Figure 3.2: Mean individuals (\pm S.D.) from the August, October, November and December samples at the Causeway (CW), Channel (CH), Raupo Bay (RB), Ebb Tide Street (ES), Penguin Street (PS), Tern Street (TS) and McCormacks Bay (MB) (Fig. 3.1).

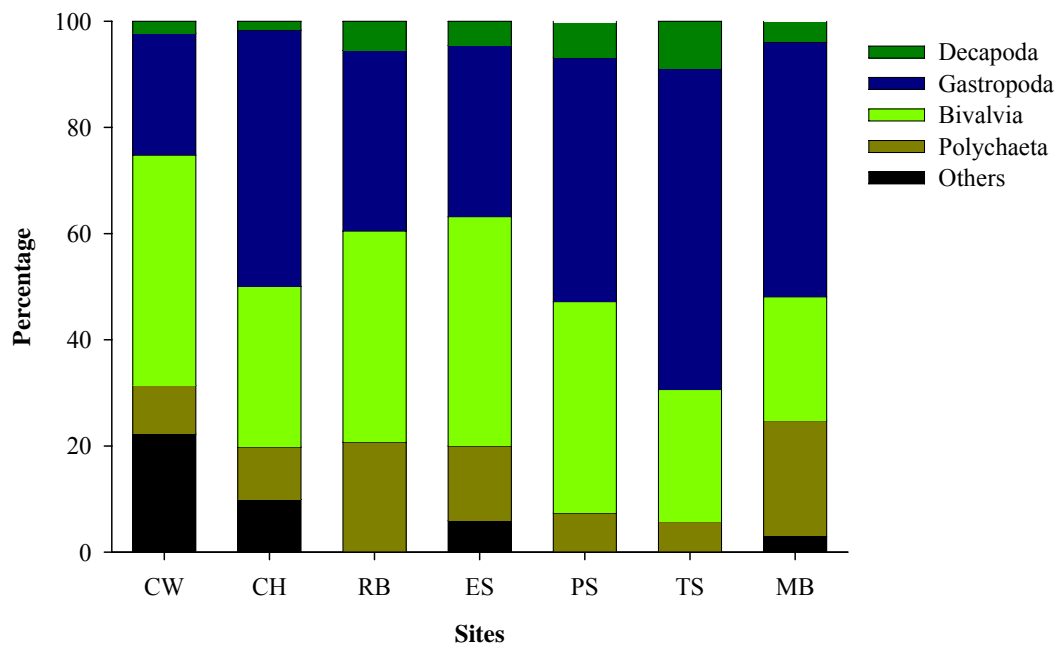


Figure 3.3: Percentage of major taxonomic groups (Decapoda, Gastropoda, Bivalvia, Polychaeta and others including: Polyplacophora, and Anthozoa) from the August, October, November and December samples at the Causeway (CW), Channel (CH), Raupo Bay (RB), Ebb Tide Street (ES), Penguin Street (PS), Tern Street (TS) and McCormacks Bay (MB) (Fig. 3.1).

Number of species at each site varied seasonally (Fig. 3.4), and there were variations in abundance. Variations in abundance of the three most common species are summarised below. Complete data on species abundance at each site and time can be found in Appendix 1.

A. stutchburyi was widely distributed and in highest abundance at the causeway sites CW and CH and ranged from 103 to 369 and 155 to 230 individuals respectively. At site CW the highest numbers were in October and at site CH in December. The lowest abundance was at site MB and ranged from 5 to 34 individuals with the highest numbers in December. *M. tenebrosus* was in highest abundance at sites CH, close to the causeway, and TS on the eastern side of the Estuary closest to the mouth. These ranged from 30 to 452 and 22 to 251 individuals respectively. At both sites CH and CW the highest numbers were in October and the lowest in August. The lowest abundance was at site MB and ranged from 0 to 2 individuals. *M. tenebrosus* was present at all sites except Site RB. *A. crenata* were in highest abundance at sites PS and TS in the eastern side of the Estuary. They ranged from 32 to 250 and 9 to 110 respectively and were in highest abundance at both sites during August. They were in lowest abundance at site PS in October and in December at site TS.

There was no clear pattern in species abundance over time. However, numbers of species increased from August through to December at sites CH and PS. At sites ES and TS there was an increase in species numbers during October followed by a decrease in November.

Rare species such as *X. plebeius* which ranged from 2 to 3 individuals, *E. modestus* ranged from 3 to 19 individuals and *A. glaucus* ranged from 2 to 3 individuals, and were not widely distributed and were absent at particular sampling times. These species were found at site CH that had the highest species richness (Table 3.2). Other species such as *Paphies australis* were well distributed (present at all sites except site MB) (Table 3.1), in low abundance (ranged from 1 at site ES to 61 at site PS) and only found once or twice in sites at sampling times as indicated by species increases and decreases at sites over time (Fig. 3.4).

Similarities in community composition were consistent between groups of sites over time (Fig. 3.5). The groupings reflected the positions of sites within the Estuary with exception of the two Bay sites MB and RB (Fig. 3.1). Sites in the west side of the Estuary, CW and CH, were grouped together ranging in community similarity from 66 to 80%. Similarity decreased in October followed by an increase in November and a decrease again in December. Grouping of the two Bay sites, MB and RB from the west and east sides of the Estuary respectively, ranged in community similarity from 51 to 66% with similarity remaining consistent between August and December, and October and November. In August

and December sites close together on the east side of the Estuary by Brighton Spit, ES and TS, were similar in community composition and together less similar to site PS in the same area. In October and November this changed to sites PS and TS becoming similar in community composition and together less similar to site ES.

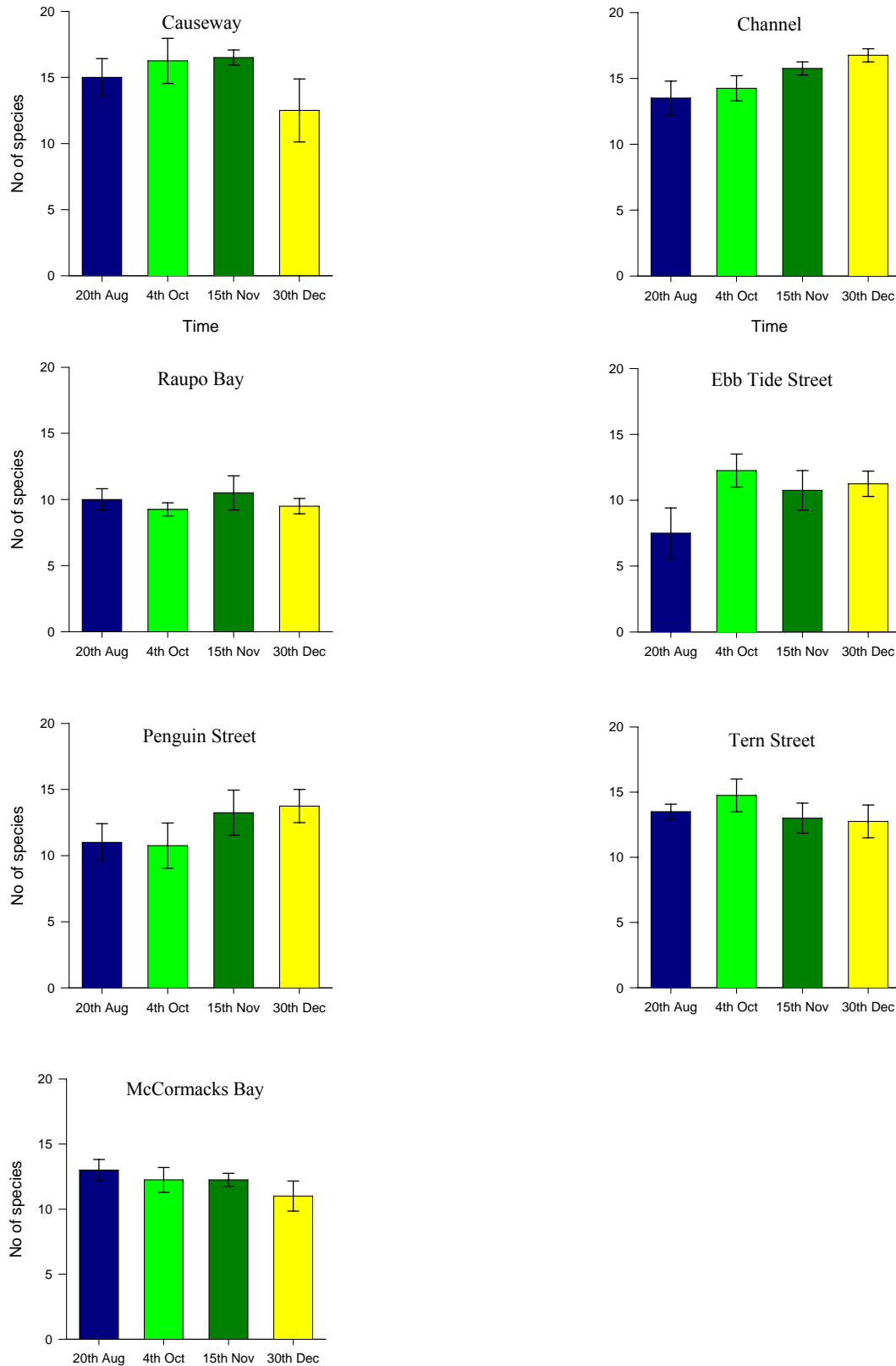
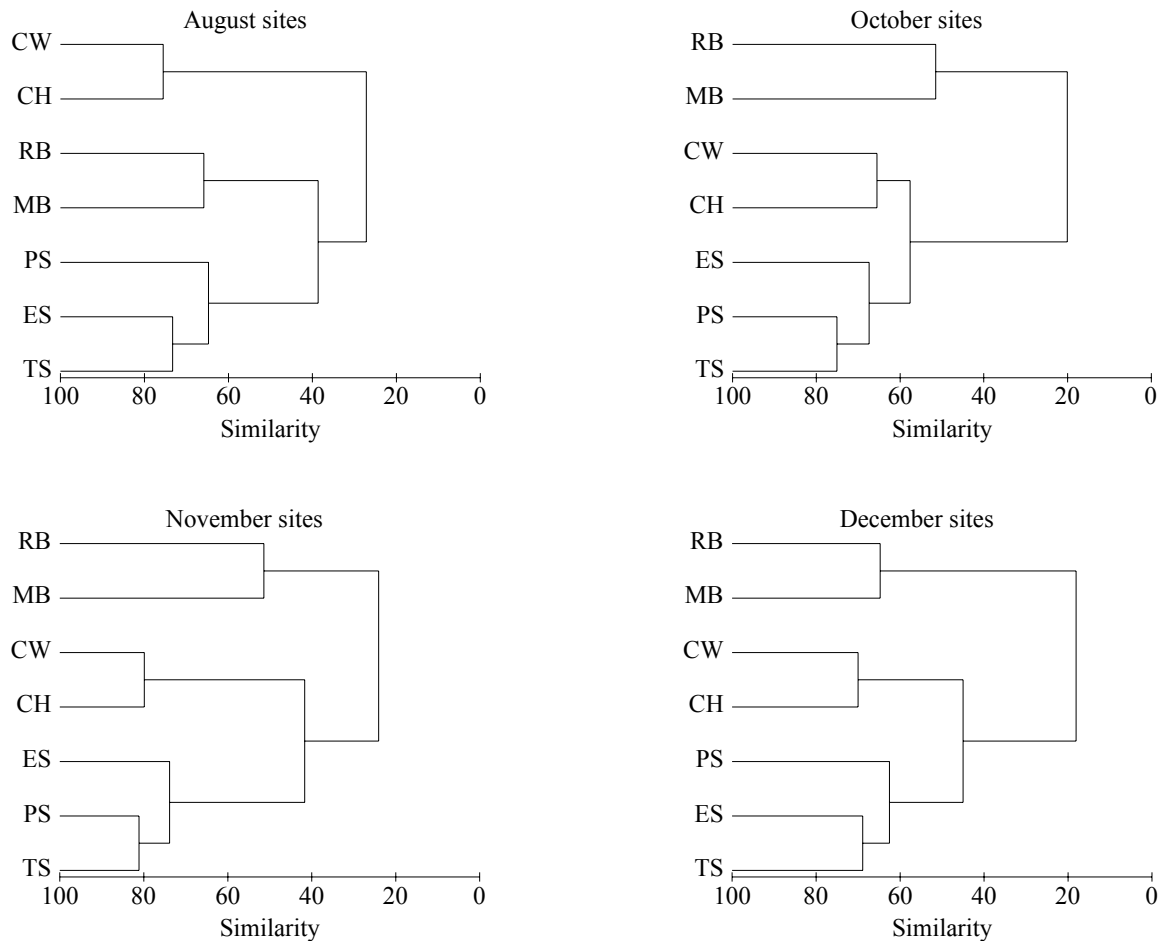


Figure 3.4: Mean (\pm S.D.) for the number of species at each sampling site and month.



Community composition changed significantly over time at each site (Table 3.3; Fig. 3.6). Community composition in August and October was similar at sites CW and RB (Fig. 3.6). The August and November communities at sites CH, ES, PS, and TS were grouped separately from the October and December communities (Fig. 3.6). In August and November, communities at sites ES and TS were grouped closer than at sites CH and PS. Similarities between the October and December communities were greater between sites CH and PS than at sites ES and TS. At all sites August and December communities were dissimilar with exception of site MB. Similar patterns of changes to community composition were between sites TS and ES (Fig. 3.6).

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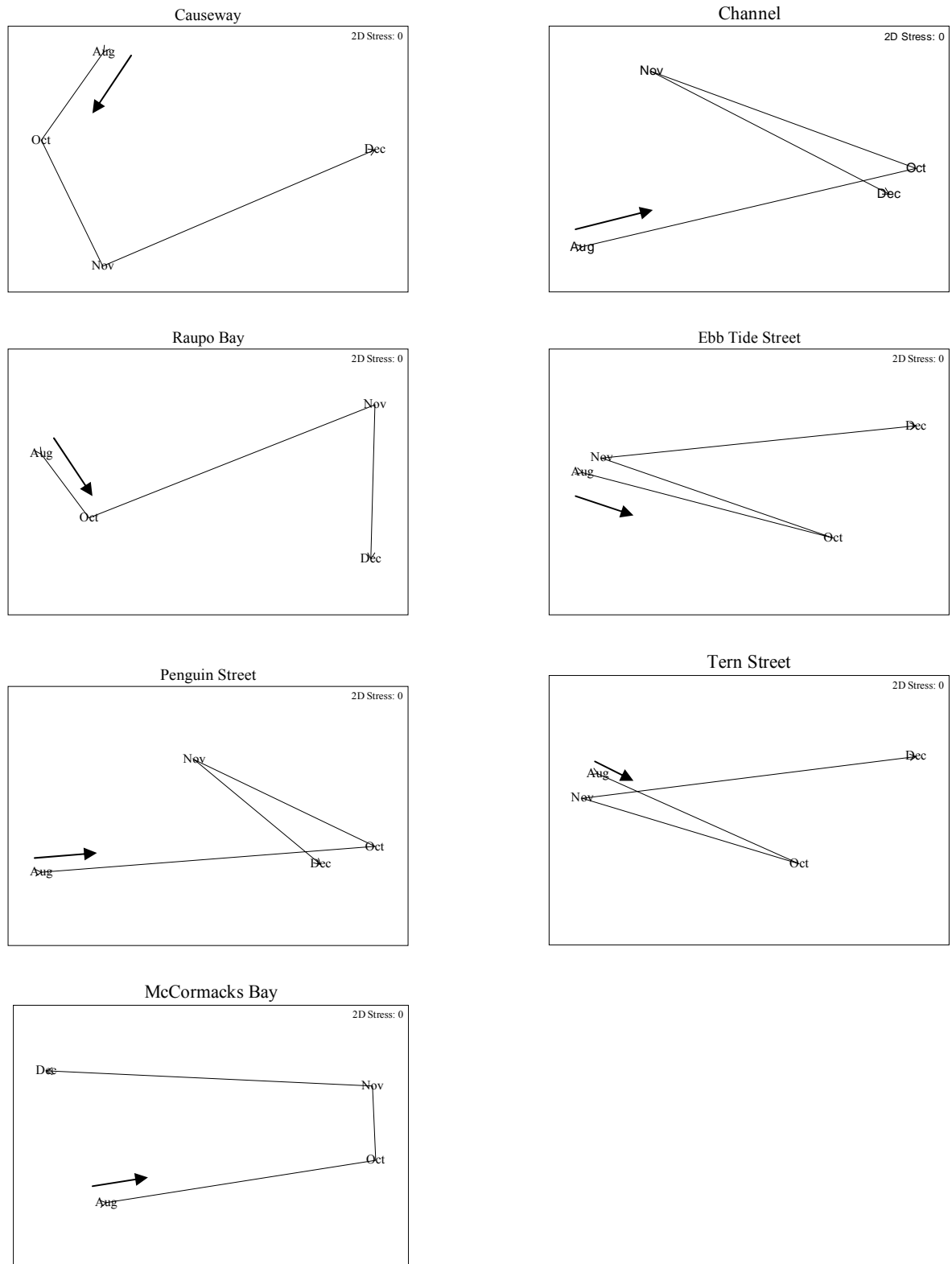


Figure 3.6: Non-metric multidimensional scaling (nMDS) ordination of mean ($n = 4$) community composition for all sites over time based on square-root-transformed abundances and Bray-Curtis similarities. Arrows indicate the start of sampling.

At each site species that cumulatively contributed to 50% of change in community composition between August (winter) and December (summer) either increased or decreased in abundance. Some species were found to be absent or present in samples from winter or summer (Table 3.4). The only clear trends were at sites CH and RB. At site CH *M. tenebrosus*, *N. helmsi* and *A. aureoradiata* increased in abundance. *Diloma nigerrima* was only present in December. *A. bifurca*, *P. estuarinus* and *Halicarcinus whitei* all decreased in abundance at site RB. *H. whitei* were not found in the December samples (Table 3.4). At the remaining sites there were no clear trends between winter and summer.

Table 3.4: The mean change in abundance of invertebrate species (positive and negative) at the Causeway, Channel, Raupo Bay, Ebb Tide Street, Penguin Street, Tern Street and McCormacks Bay sites between the winter (August) of 2005 and summer (December) of 2005. Species that cumulatively contribute to 50% of the variations between seasons are shown.

| Causeway | Aug | Dec | Channel | Aug | Dec |
|---------------------------------|-----------------|-----------------|---------------------------------|-----------------|-----------------|
| Species | Av.Abun. | Av.Abun. | Species | Av.Abun. | Av.Abun. |
| <i>Diloma nigerrima</i> | 0 | 4 | <i>Micrelenchus tenebrosus</i> | 2.27 | 9.55 |
| <i>Diloma subrostrata</i> | 2.38 | 0 | <i>Notoacmea helmsi</i> | 2.07 | 7.07 |
| <i>Macomona liliana</i> | 4.75 | 6.88 | <i>Anthopleura aureoradiata</i> | 2.42 | 5.5 |
| <i>Austrovenus stutchburyi</i> | 6.88 | 5.06 | <i>Diloma nigerrima</i> | 0 | 2.48 |
| Raupo Bay | | | Ebb Tide Street | | |
| <i>Arthritica bifurca</i> | 6.63 | 2.94 | <i>Macomona liliana</i> | 0.75 | 4.42 |
| <i>Potamopyrgus estuarinus</i> | 2.92 | 3.95 | <i>Amphibola crenata</i> | 3.45 | 0.25 |
| <i>Halicarcinus whitei</i> | 0.75 | 0 | <i>Anthopleura aureoradiata</i> | 0 | 2.72 |
| Penguin Street | | | Tern Street | | |
| <i>Amphibola crenata</i> | 7.9 | 3.7 | <i>Amphibola crenata</i> | 5.2 | 1.25 |
| <i>Paphies australis</i> | 3.89 | 0 | <i>Micrelenchus tenebrosus</i> | 2.27 | 5.39 |
| <i>Arthritica bifurca</i> | 5.29 | 2.02 | <i>Diloma nigerrima</i> | 0 | 2.51 |
| <i>Diloma nigerrima</i> | 0.25 | 2.95 | <i>Notoacmea helmsi</i> | 1.39 | 3.52 |
| McCormacks Bay | | | | | |
| <i>Austrovenus stutchburyi</i> | 1.1 | 2.88 | | | |
| <i>Boccardia polybranchia</i> | 1.99 | 0.5 | | | |
| <i>Helice crassa</i> | 1.97 | 0.56 | | | |
| <i>Arthritica bifurca</i> | 3.86 | 5.17 | | | |
| <i>Anthopleura aureoradiata</i> | 1.29 | 0 | | | |

At each site there were correlations between individual species from the total assemblages and *U. lactuca* biomass (Table 3.5). At site CH five species were positively correlated with *U. lactuca* biomass. These were: decapods *H. crassa* and *H. whitei*, the gastropod *N. helmsi*, the polychaete *Heteromastus filiformis* and the anthozoan *A.*

aureoradiata (Table 3.5). There were no negative correlations at this site. There were two species positively correlated with *U. lactuca* biomass at site RB: *Paphies australis* and *Nicon aestuariensis*. The gastropod *A. crenata* was the only species negatively correlated with *U. lactuca* biomass at site ES; the decapod *H. whitei*, the gastropod *N. helmsi*, the bivalve *M. liliana*, the polychaete *S. benhami* and the anthozoan *A. aureoradiata* were all positively correlated with *U. lactuca* biomass (Table 3.5). At site MB the gastropod *Zeacumantus lutulentus* was positively correlated and the polychaete *S. benhami* was negatively correlated with *U. lactuca* spp. biomass. At site CW *Diloma nigerrima*, *M. liliana* and *H. filiformis* were positively correlated and *Diloma subrostrata* and *A. stutchburyi* were negatively correlated with *U. lactuca* spp. biomass. At site TS, *H. crassa*, *Macrophthalmus hirtipes* and *Scoloplos cylindrifer* were positively correlated and *A. crenata*, *A. bifurca* and *H. filiformis* were negatively correlated with *U. lactuca* spp. biomass (Table 3.5).

Table 3.5: Pearsons correlation (r^2) results between *Ulva lactuca* biomass (dry wt. g) and invertebrate species at the Causeway, Channel, Raupo Bay, Ebb Tide Street, Penguin Street, Tern Street and McCormacks Bay (Fig. 3.1) for the August, October, November and December samples. There was no *Ulva lactuca* in any of the samples at Penguin Street.

| Causeway | | Channel | |
|--------------------------------|---|---------------------------------|---|
| Species | Pearson's correlation coefficient (r^2) | Species | Pearson's correlation coefficient (r^2) |
| <i>Diloma nigerrima</i> | 0.677** | <i>Helice crassa</i> | 0.675** |
| <i>Diloma subrostrata</i> | -0.852** | <i>Halicarcinus whitei</i> | 0.540** |
| <i>Austrovenus stutchburyi</i> | -0.815** | <i>Notoacmea helmsi</i> | 0.760** |
| <i>Macomona liliana</i> | 0.782** | <i>Anthopleura aureoradiata</i> | 0.832** |
| <i>Heteromastus filiformis</i> | 0.623** | <i>Heteromastus filiformis</i> | 0.904** |
| Raupo Bay | | Ebb Tide Street | |
| <i>Paphies australis</i> | 0.598** | <i>Halicarcinus whitei</i> | 0.678** |
| <i>Nicon aestuariensis</i> | 0.598** | <i>Amphibola crenata</i> | -0.684** |
| | | <i>Macomona liliana</i> | 0.556** |
| | | <i>Notoacmea helmsi</i> | 0.504** |
| | | <i>Scolecopides benhami</i> | 0.756** |
| | | <i>Anthopleura aureoradiata</i> | 0.750** |
| Tern Street | | McCormacks Bay | |
| <i>Helice crassa</i> | 0.726** | <i>Zeacumantus lutulentus</i> | 0.506* |
| <i>Macrophthalmus hirtipes</i> | 0.747** | <i>Scolecopides benhami</i> | -0.549* |
| <i>Amphibola crenata</i> | -0.728** | | |
| <i>Arthritica bifurca</i> | -0.715** | | |
| <i>Heteromastus filiformis</i> | -0.577* | | |
| <i>Scoloplos cylindrifer</i> | 0.759** | | |

* Correlation is significant (α) = 0.05 (2-tailed)

**Correlation is significant (α) = 0.01 (2-tailed)

3.2.2 Physico-chemical variables and *Ulva lactuca* biomass

There were variations in physico-chemical variables and *U. lactuca* biomass within and between sites and sampling times. These are described below and summarised in Figure 3.7.

Dissolved oxygen in sediment pore water varied over time. Variations differed significantly between sites (ANOVA, $F=51.00$ ($df=6, 21$) $p < 0.001$). Levels were highest at site CW, and ranged from 12.6 to 12.2 mg/l^{-1} , and levels were lowest at site MB, ranging from 3.0 to 3.3 mg/l^{-1} . Site RB had greatest variations over time dropping from 8.6 mg/l^{-1} in August to 6.6 mg/l^{-1} in December. There was a small peak at site CH in November; dissolved oxygen increased to 10.7 mg/l^{-1} and then dropped in December to 9.9 mg/l^{-1} .

Temperature of pore water increased steadily between August in winter and December in summer at all sites. In August, temperature ranged from 14.3°C, recorded at site RB, to 18.4°C at site MB. By December, temperature ranged from 22.8 °C recorded at site CW to 26.1°C at site TS, which had a similar temperature to site PS. There was little variation between sites at any other time.

Salinity of pore water fluctuated over time at each site and varied significantly between sites (ANOVA, $F=86.47$, ($df=6, 21$) $p < 0.001$). Highest readings were from site TS close to the Estuary mouth (Fig. 3.1) varying between 31 and 35 ppt over sampling times. Lowest readings were at site RB furthest from the Estuary mouth and closest to the Avon River (Fig. 3.1). These varied between 22.1 and 26.3 ppt. Greatest variation in salinity over time occurred during October at sites CW and MB when salinity dropped by ~50% followed by an increase in levels in November. Most similar readings over time and between sites were from sites ES and PS.

Percentage of sediment pore water fluctuated over time and varied significantly between sites (ANOVA, $F=28.04$ ($df=6, 21$) $p < 0.001$) with no clear pattern. Overall lowest levels at each site were in November and varied between sites. Site PS had the lowest percentage at 21.4% and the highest was at site RB with 31.2%. The highest percentages of sediment pore water occurred at sites RB, TS and MB and the lowest were at sites ES and PS.

Percentage of organics varied significantly between sites and over time with no clear trend (ANOVA, $F=97.4$ ($df=6, 21$) $p < 0.001$). The range was between 1.22 at site ES and 5.78% at site MB. Sites RB and MB had the highest percentages and ranged from 3.5 to 4.5 and 3.1 to 5.78% respectively. Sites CW and CH were the most similar with ranges between 2.4 and 3.1%, and 2.5 and 2.9% respectively. Sites ES and PS had the lowest percentages of organics. These sites' highest levels were in October, reaching 2.2% and 2.0% respectively.

Percentage of silt/clay varied significantly over time and between sites with no clear pattern (ANOVA, $F=5.67$ ($df=6, 21$) $p < 0.001$). Sites RB and MB had the highest percentage of silt/clay ranging between 67.7 and 75.2, and 65.1 and 74.7% respectively. Site PS had the lowest percentage of silt and clay with a peak in October of 14.69% and ranged between 3.20% and 8.25% for other months. Sites CW, ES and TS were similar. These sites had low percentages of silt/clay in August, rising in October, with variations in November and December. Site CH's silt/clay fraction dropped from 13.1% in August to 0.4% in October followed by an increase in November and December to 12.9% and 16.1% respectively.

U. lactuca biomass varied significantly over time and between sites with an increase between August in the winter and December in the summer (ANOVA, $F=18.4$ ($df=6, 21$) $p < 0.001$). Sites CW and CH had the highest biomass of *U. lactuca* and ranged from 2.4 in August to 21.8g in December and 0 in August to 21.8 g in December respectively. Site PS had no biomass of *U. lactuca* in August or December. At site MB there was a peak in *U. lactuca* biomass during October. Biomass ranged from 3.7 to 12.3 g at this time. In December biomass was low compared to the other sites at this time and ranged from 1.8 to 2.4 g.

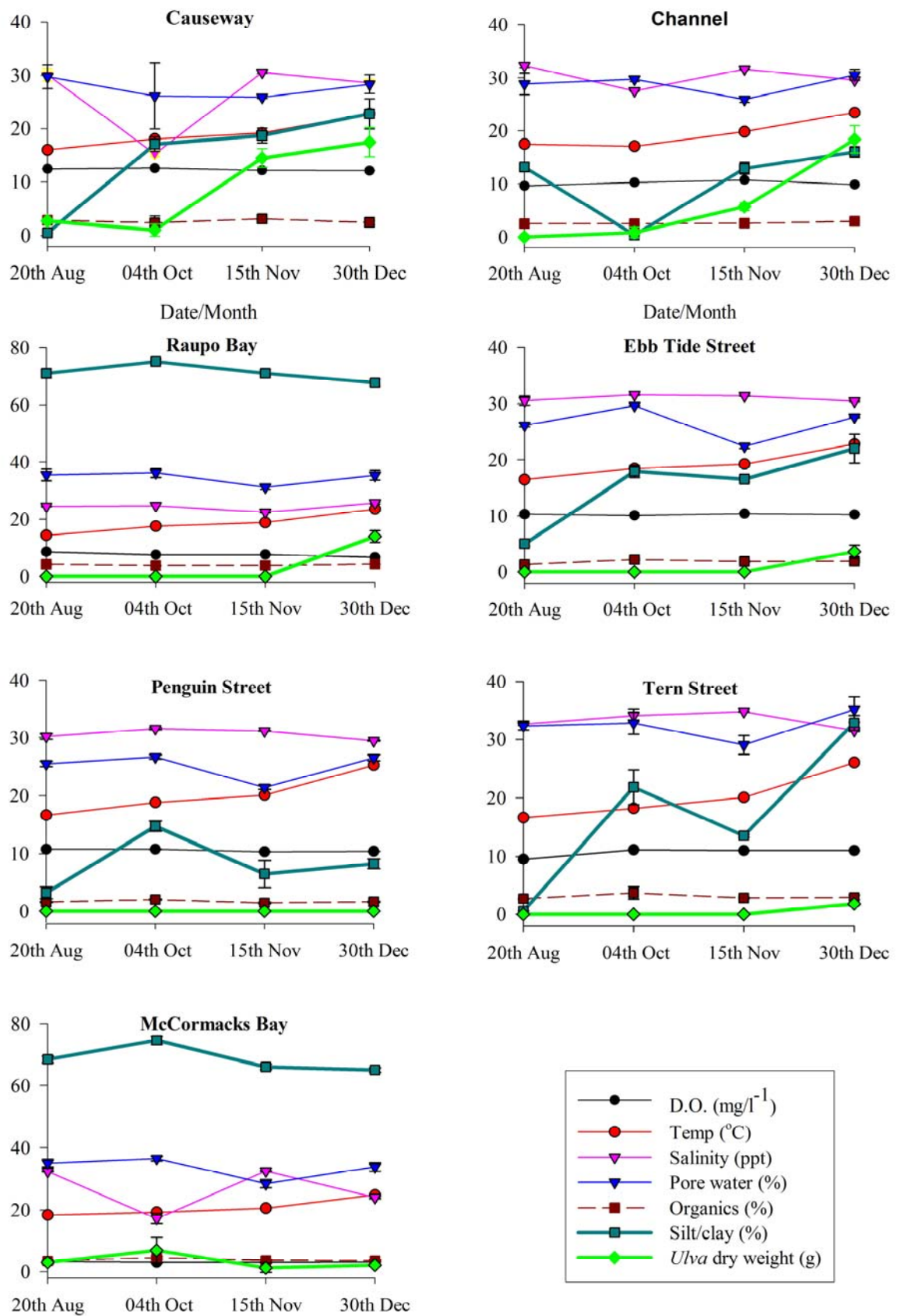


Figure 3.7: Mean (±S.D.) physico-chemical variables and *Ulva lactuca* biomass for all sites and sampling times during the general survey.

There were trends in similarity of physico-chemical variables between groups of sites over time. These are summarised in (Fig. 3.8). Sites RB and MB were grouped together over all sampling times and became more similar in December. In August, October and November, sites PS and ES were linked together. In December site ES became closely linked to site CW and was no longer closely linked to site PS. Sites CH, TS, and CW remained grouped together over time with variation between site linkages. Site CW had the most dissimilar linkage to this group in October.

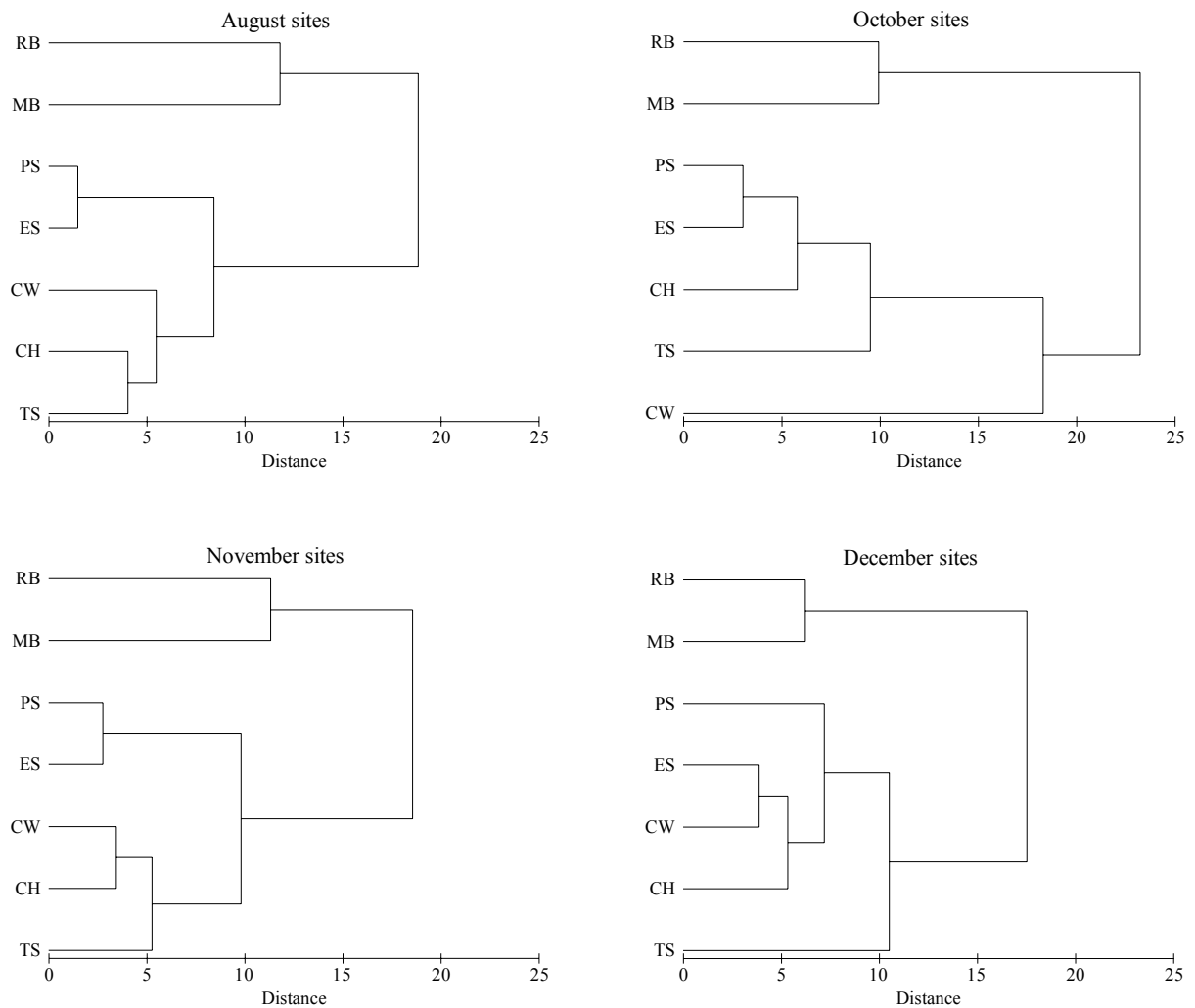


Figure 3.8: Dendrograms of similarities in physico-chemical variables between sites and sampling times.

The results of PCA ordinations were accurate representations of patterns of changes in physico-chemical variables at each site over time (Fig 3.9). Accuracy was shown by high percentages of variation explained by the PC1 and PC2 axes (Table 3.6). At each site there were significant variations in combinations of physico-chemical variables over time (Table 3.7). Significance of individual variables driving the dissimilarity at each site over time is

represented by the length of blue vector lines approaching the blue circle (Fig. 3.9). These changes are described below and summarised in Figure 3.9.

At sites CW and MB the most significant variables driving dissimilarity between sampling times were sediment temperature and salinity. Dissolved oxygen was the least significant variable. There were three variables of significance at site CH. The main variable was sediment temperature, followed by equal contributions from salinity and pore water. The least significant variable was dissolved oxygen. Temperature and pore water were equally significant at sites RB, ES, and PS. Silt/clay, salinity and dissolved oxygen were, in decreasing order, the least significant variables at each of these sites. Temperature and organic matter were equally the most significant variables at sites TS with salinity being least significant.

At site CW the physico-chemical samples were most similar in November and December and closely linked to August samples. At sites RB and PS the August and October samples were most similar. These were linked closely to the November samples. At site MB October had the least similar samples to other times. Samples in August and November were the most similar. Site CH had the least variation between samples over time. August and November samples were closely linked to October samples; all three were closely linked to the December samples. At site ES the August and November, and October and December samples were most similar. The October and November samples were closely linked to the August samples at site TS. At this site the December samples were most dissimilar to any of the other sampling times.

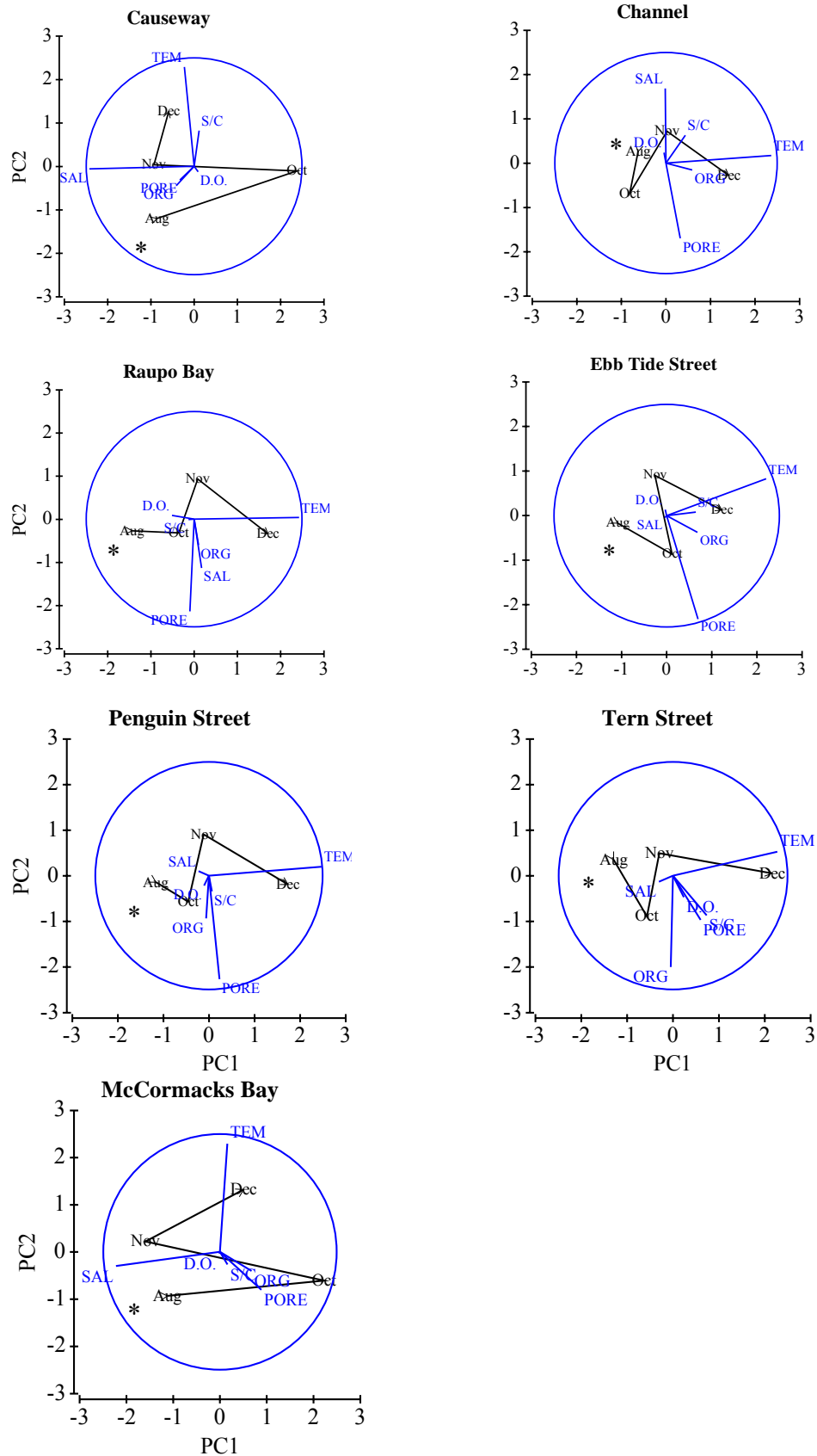


Figure 3.9: PCA ordinations of physico-chemical variables: dissolved oxygen (DO), temperature (TEM), pore water (PORE), salinity (SAL), volatile solids (ORG), and silt/clay (S/C) in August, October, November, and December at all sites. Blue circles and lines represent the variable vectors and their contribution to dissimilarity between times of sampling. Asterisk (*) indicates the first sample time.

Table 3.6: The total variation accounted for by PC1 and PC2 in physico-chemical variables for each site over time.

| Axis | CW | CH | RB | Sites ES | PS | TS | MB |
|-----------------|------|------|------|-------------|------|------|------|
| PC1 | 67.7 | 67.0 | 78.6 | 58.0 | 76.1 | 76.5 | 68.8 |
| PC2 | 26.8 | 24.9 | 17.8 | 34.2 | 20.1 | 13.3 | 23.1 |
| Total variation | 94.5 | 91.9 | 96.4 | 92.2 | 96.2 | 89.8 | 91.9 |

Table 3.7: Global R and p-values for the similarity of physico-chemical variables between sampling times at each site.

| | CW | CH | RB | ES | PS | TS | MB |
|-----------------|---------|---------|----------|---------|---------|----------|----------|
| Similarity, R | =0.999, | =0.995, | R=0.986, | R=1, | R=1, | R=0.840, | R=0.802, |
| Significance, p | <0.001 | p<0.001 | p<0.001 | p<0.001 | P<0.001 | p<0.001 | p<0.001 |

Results of BIOENV analysis (Chapter 2, Section 2.4) showed the silt/clay fraction to be, overall, the most important, physico-chemical variable correlated with patterns of invertebrate assemblages during August (Table 3.8). However, the low Rho-value of 0.209 (Rho is the rank correlation coefficient; closer to 1 = high correlation, closer to 0 = low correlation) showed this relationship was a weak correlation. In October the correlation was much stronger (Rho = 0.775) and three physico-chemical variables (temperature, pore water and the silt/clay fraction) were best correlated with patterns of invertebrate assemblages shown in Figure 3.6. In November dissolved oxygen of the sediment pore water was the best correlated variable (Rho = 0.699). By December a combination of dissolved oxygen, organics and silt/clay were the best correlated variables (Rho = 0.734) to community assemblage patterns. All assemblages at individual sites showed variation over time (Fig. 3.6). BIOENV analysis showed that at site CW dissolved oxygen and organics correlated best with patterns of assemblages over time (Table 3.9). At site CH salinity was the most strongly correlated variable (Rho = 0.771; Table 3.9). At the remaining sites, in various combinations, temperature, pore water, silt/clay fraction and organics were all highly correlated with patterns of assemblages over time (Table 3.9). The groups of variables showing the strongest correlation with patterns of assemblages (Rho = 0.943) was at site TS. The group included: temperature, pore water, organics and the silt/clay fraction (Table 3.9). Over all sites and variables the weakest correlation was in temperature at site MB (Rho = 0.314; Table 3.9). *U. lactuca* dry weight biomass did not feature in any BIOENV results.

Table 3.8: Results of the BIOENV analysis. The Rho-value indicates the level of correlation of environmental variables that fit best with patterns in community structure from all sites during each month.

| Sampling date/month | Environmental variables | Rho-value |
|---------------------------|-------------------------------|-----------|
| 20 th August | Silt/clay | 0.209 |
| 04 th October | Temp + pore water + silt/clay | 0.775 |
| 15 th November | D. O. | 0.699 |
| 30 th December | D.O. + organics + silt/clay | 0.734 |

Table 3.9: Results of the BIOENV analysis for individual sites and the physico-chemical variables that correlated best with the patterns in community structure (refer to Fig. 3.6).

| Site | Environmental variables | Rho-value |
|-----------------|---------------------------------------|-----------|
| Causeway | D.O and organics | 0.657 |
| Channel | Salinity | 0.771 |
| Raupo Bay | Temp and pore water | 0.771 |
| Ebb Tide Street | Temp and organics | 0.657 |
| Penguin Street | Temp, pore water, silt/clay | 0.714 |
| Tern Street | Temp, pore water, organics, silt/clay | 0.943 |
| McCormacks Bay | Temp | 0.314 |

Size and Condition Indices (CI) of Austrovenus stutchburyi

There were variations in the size and Condition Indices (CI) of *A. stutchburyi* between sites. The main differences are described below and summarised in Figure 3.10. Complete data on the size and condition of *A. stutchburyi* at each site and time can be found in Appendix 2.

The largest individuals were at the CH site close to the causeway and Heathcote channel and ranged from 20.1 mm in August to 47.2 mm in October. The condition was best in August, CI ranged from 3.5 to 6.0 and worst in December, CI ranged from 3.4 to 3.9. The smallest individuals were at site TS closest to the Estuary mouth and ranged from 11.1 mm in November to 28.9 mm in December. The condition was best in August, CI ranged from 5.5 to 7.5 and worst in December, CI ranged from 4.4 to 6.7. Between the seven sites, condition of *A. stutchburyi* was best at site TS closest to the Estuary mouth and worst at CH near to the causeway and beside the Heathcote channel. At site CH, abundance of *A. stutchburyi* ranged from 568 to 816 individuals and was greatest in October and least in December. At site TS, the range was 180 to 352 individuals and was greatest in December and least in November.

There were significant variations in condition indices of *A. stutchburyi* between sites in each month (Fig. 3.10 and Table 3.10). No significant variations were found in condition

indices at individual sites over time (Table 3.11). There were no positive or negative correlations between condition indices and physico-chemical variables at any site or time.

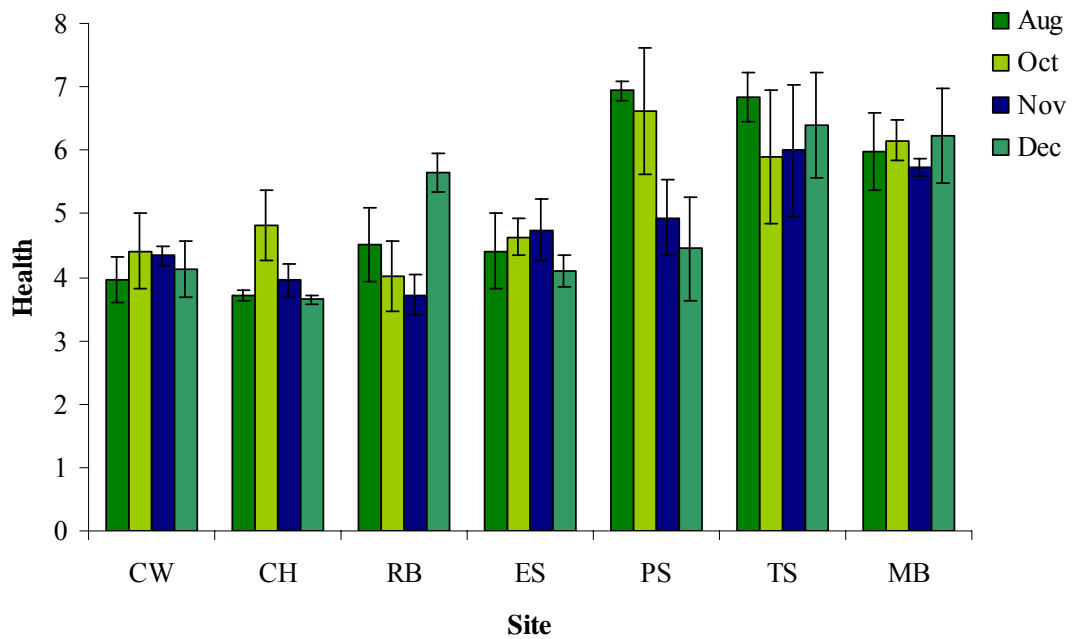


Figure 3.10: Mean (\pm S.E.) Condition Index (CI) of *Austrovenus stutchburyi* at sites CW, CH, RB, ES, PS, TS, and MB in August, October, November and December.

Table 3.10: Summary ANOVA results of the variance in the Condition Index (CI) of *Austrovenus stutchburyi* between sites CW, CH, RB, ES, PS, TS and MB in August, October, November and December.

| Month | F | p-value |
|----------|--------|---------|
| August | 10.247 | < 0.001 |
| October | 2.825 | < 0.05 |
| November | 4.825 | < 0.005 |
| December | 5.067 | < 0.005 |

Table 3.11: Summary ANOVA results of the variance in the Condition Index (CI) of *Austrovenus stutchburyi* over time (August, October, November and December) at sites CW, CH, RB, ES, PS, TS, and MB.

| Site | F | p-value |
|------|-------|---------|
| CH | 0.244 | 0.864 |
| CW | 3.108 | 0.067 |
| RB | 3.532 | 0.048 |
| ES | 0.438 | 0.730 |
| PS | 2.943 | 0.076 |
| TS | 0.949 | 0.448 |
| MB | 0.181 | 0.907 |

3.2.3 Comparisons between total individuals and taxa recorded in 2003 and the present study

Comparisons were made between the mean proportions of total individuals and taxa from the combined community assemblages at sites CW, CH, RB, ES, PS, and TS from August, October, November, and December in the present study and Bressington's in (2003). Total proportions of individuals were different in the groups of Bivalvia, Gastropoda, Decapoda and others (Polychaeta and Anthozoa) (Fig 3.11). Compared with 2003, proportions of total individuals were 16 % higher in 2005 for Bivalvia, 16.8% lower for Gastropoda, 2.2% lower for Decapoda and 6% higher for the other groups (Fig. 3.11). Proportions of total taxa were different in all the groups. Bivalvia taxa were 9% higher in 2005 compared to 2003, Gastropoda were 12.4% lower and Decapoda and other taxa were 2.2% and 1.2% higher respectively (Fig. 3.12).

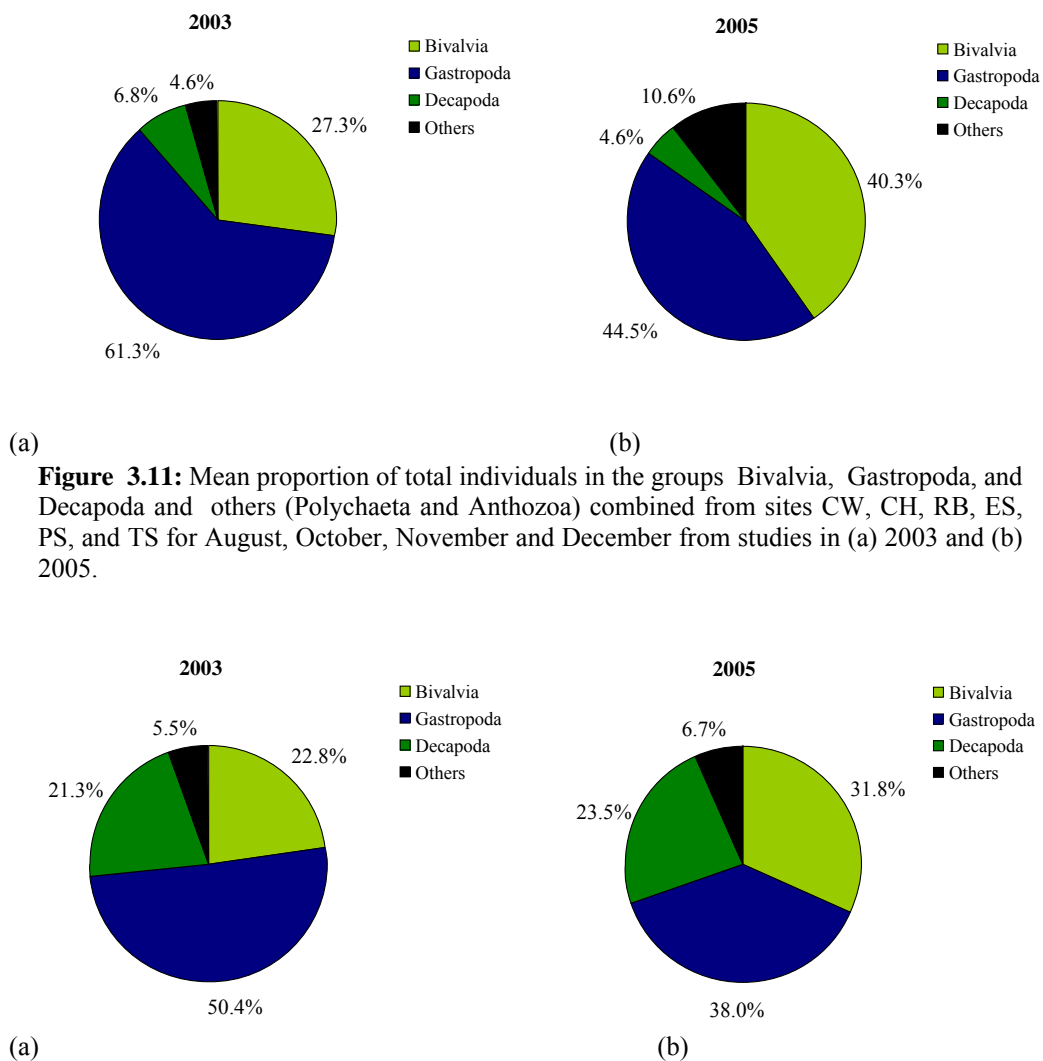


Figure 3.11: Mean proportion of total individuals in the groups Bivalvia, Gastropoda, and Decapoda and others (Polychaeta and Anthozoa) combined from sites CW, CH, RB, ES, PS, and TS for August, October, November and December from studies in (a) 2003 and (b) 2005.

Figure 3.12: Mean proportion of taxa in the groups Bivalvia, Gastropoda, Decapoda and others (Polychaeta and Anthozoa) combined from the August, October, November and December samples in sites CW, CH, RB, ES, PS, and TS from studies in (a) 2003 and (b) 2005.

3.2.4 Comparisons of community assemblages between December 2003 and the present study

Comparisons were made between the means of community assemblages recorded from sites CW, CH, RB, ES, PS, and TS in 2003 and 2005. Differences were found in number of individuals and species recorded during each study. Differences between communities from the studies are described below and summarised in Figure 3.13 and Table 3.12.

In December of 2003, 19 species were recorded in five groups (Decapoda, Gastropoda, Bivalvia, Polychaeta and others- Polyplacophora and Anthozoa) compared to 27 species in 2005; percentage of these taxonomic groups was different at each site between years with exception of site TS (Fig. 3.13). Site CW was dominated by gastropods in 2003. In contrast there were even distributions of gastropods, bivalves and polychaetes in 2005. Polychaetes dominated at site CH in 2003 compared to dominance of gastropods in 2005. At site RB decapods and gastropods were the main groups in 2003 compared to polychaetes in 2005. Polychaetes dominated at site ES in 2003 but in 2005 polychaetes and bivalves were dominant. Decapods and polychaetes dominated at site PS in 2003. In 2005 gastropods and bivalves were dominant. Furthermore, in December 2005 there was greater mean numbers of individuals per square meter at all sites compared to 2003 (Table 3.12). The greatest difference occurred at sites RB and CW. Number of individuals were greater by 1335 per m² and 1219 per m² respectively with five more species at each site. Site PS had the next highest difference with 1147 more individuals per m² and five more species. At site CW the assemblages differed by a mean of 1219 individuals and five more species. The lowest difference was at site ES with 288 more individuals and six more species. The greatest difference in number of species was at site CH with seven new species and 867 more individuals per m². Site TS had the lowest increase in species with two new species and 760 more individuals per m².

MDS representation of the two benthic invertebrate assemblages in December 2003 and 2005 at sites CW, CH, RB, ES, PS, and TS was highly accurate with a low 2D stress of 0.09 (low stress levels reflect high accuracy of the represented data on the ordination) (Fig. 3.14). There was similarity between the compared communities at each site as reflected by the low ANOSIM R-value of 0.139. However, similarities were not significant ANOSIM $p=0.087$.

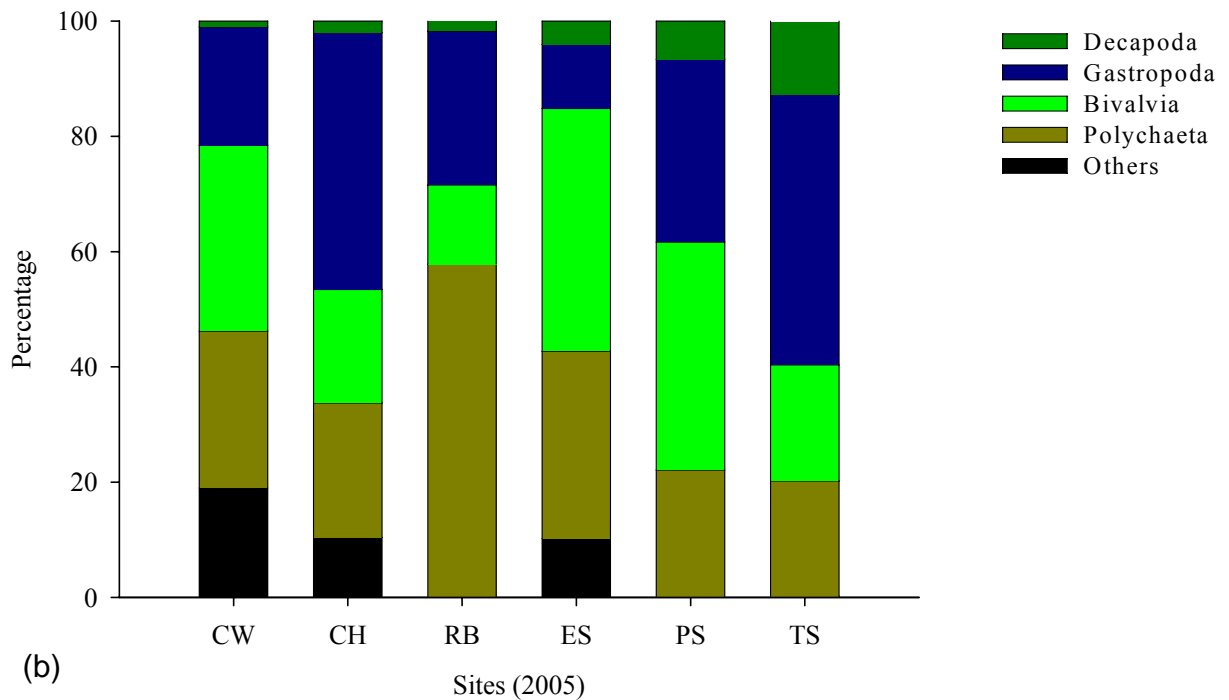
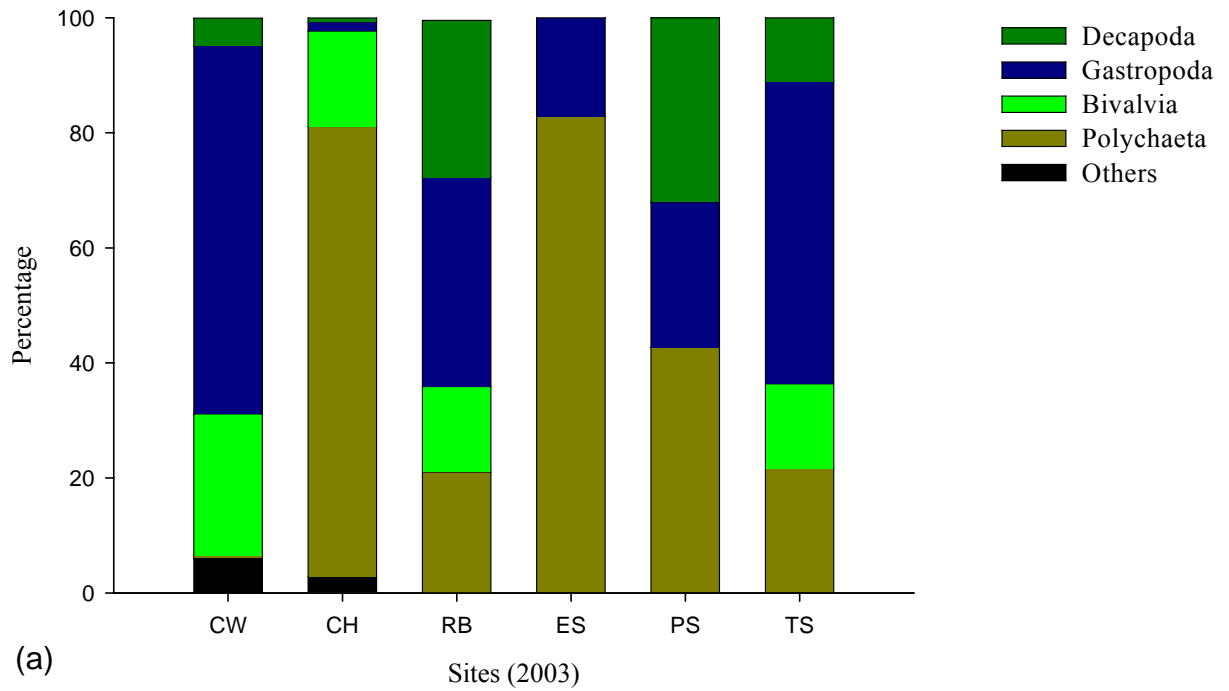


Figure 3.13: The percentage of major taxonomic groups (Decapoda, Gastropoda, Bivalvia, Polychaeta and others: Polyplacophora and Anthozoa) for the Causeway (CW), Channel (CH), Raupo Bay (RB), Ebb Tide Street (ES), Penguin Street (PS) and Tern Street (TS) (a) December 2003 and (b) December 2005.

Table 3.12: Mean benthic invertebrates per (m²), total individuals, and species richness from the Causeway (CW), Channel (CH), Raupo Bay (RB), Ebb Tide Street (ES), Penguin Street (PS) and Tern Street (TS) in December 2003 and December 2005.

| Fauna December | CW | | CH | | RB | | ES | | PS | | TS | |
|----------------------------------|-------------|-------------|-------------|-------------|------------|-------------|-------------|-------------|------------|-------------|-------------|-------------|
| | 03 | 05 | 03 | 05 | 03 | 05 | 03 | 05 | 03 | 05 | 03 | 05 |
| <i>Helice crassa</i> | 0 | 16 | 0 | 104 | 13 | 28 | 0 | 16 | 0 | 0 | 0 | 116 |
| <i>Halilcarinus whitei</i> | 13 | 8 | 31 | 4 | 0 | 0 | 0 | 4 | 13 | 0 | 13 | 40 |
| <i>Hemigrapsus crenulatus</i> | 89 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 | 31 | 0 |
| <i>Macrophthalmus hirtipes</i> | 13 | 8 | 0 | 0 | 44 | 0 | 0 | 32 | 44 | 56 | 76 | 80 |
| <i>Amphibola crenata</i> | 0 | 0 | 0 | 0 | 76 | 156 | 133 | 4 | 31 | 220 | 13 | 36 |
| <i>Micrelenchus tenebrosus</i> | 1391 | 328 | 31 | 1480 | 0 | 0 | 13 | 12 | 0 | 36 | 400 | 468 |
| <i>Diloma spp</i> | 164 | 256 | 44 | 100 | 0 | 0 | 0 | 44 | 13 | 140 | 102 | 104 |
| <i>Potamopyrgus estuarinus</i> | 0 | 0 | 0 | 0 | 0 | 256 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Notoacmea helmsi</i> | 0 | 160 | 0 | 848 | 0 | 0 | 13 | 64 | 0 | 12 | 0 | 200 |
| <i>Xymene plebeius</i> | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Cominella glandiformis</i> | 0 | 4 | 0 | 16 | 0 | 0 | 13 | 20 | 0 | 12 | 58 | 60 |
| <i>Austrovenus stutchburyi</i> | 476 | 412 | 489 | 920 | 31 | 56 | 0 | 200 | 0 | 376 | 147 | 272 |
| <i>Macomona liliana</i> | 120 | 760 | 280 | 128 | 0 | 0 | 0 | 316 | 0 | 76 | 13 | 92 |
| <i>Arthritica bifurca</i> | 0 | 0 | 0 | 32 | 0 | 140 | 0 | 28 | 0 | 72 | 0 | 4 |
| <i>Mytilus galloprovincialis</i> | 0 | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| <i>Paphies australis</i> | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Anthopleura aureoradiata</i> | 147 | 692 | 133 | 492 | 0 | 0 | 0 | 132 | 0 | 0 | 0 | 4 |
| <i>Amaurochiton glaucus</i> | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Elminius modestus</i> | 0 | 0 | 0 | 68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scolecoides benhami</i> | 0 | 25 | 13 | 140 | 44 | 268 | 76 | 115 | 31 | 25 | 102 | 51 |
| <i>Boccardia polybranchia</i> | 1 | 331 | 0 | 280 | 0 | 166 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Pectinaria australis</i> | 0 | 25 | 0 | 140 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Heteromastus filiformis</i> | 9 | 433 | 0 | 369 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Nicon aestuariensis</i> | 0 | 0 | 31 | 0 | 0 | 255 | 13 | 0 | 0 | 0 | 0 | 0 |
| <i>Scoloplos cylindrifera</i> | 2 | 153 | 342 | 357 | 0 | 0 | 667 | 280 | 13 | 268 | 58 | 217 |
| <i>Capitella sp.</i> | 0 | 0 | 0 | 0 | 0 | 204 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sipunculid worm | 0 | 26 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 102 |
| Nermertine worm | 0 | 0 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Aonides trifidus</i> | 0 | 0 | 2787 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Paraboccardia syrtis</i> | 0 | 0 | 413 | 0 | 0 | 0 | 76 | 0 | 31 | 0 | 76 | 0 |
| Mean individuals m2 | 2427 | 3646 | 4640 | 5507 | 209 | 1544 | 1004 | 1292 | 178 | 1325 | 1089 | 1849 |
| Total Species richness | 13 | 18 | 12 | 19 | 5 | 10 | 8 | 14 | 7 | 12 | 12 | 14 |

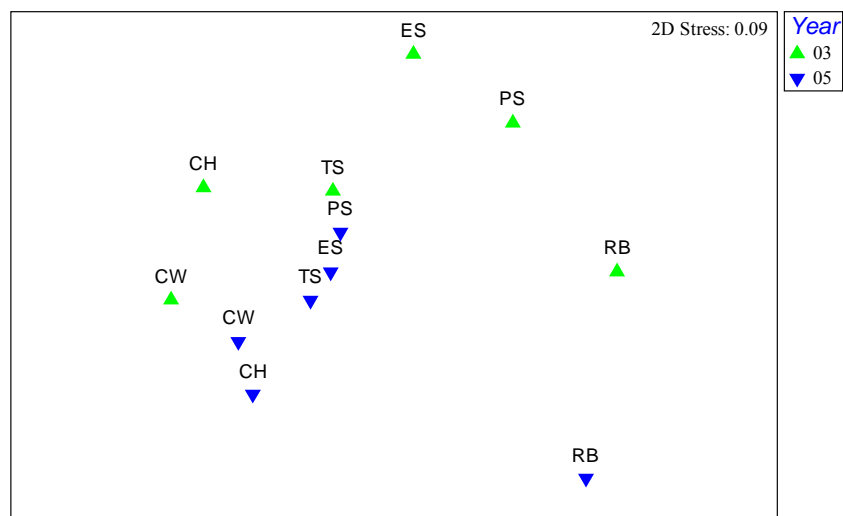


Figure 3.14: MDS ordination of invertebrate assemblage similarity between the Causeway (CW), Channel (CH), Raupo Bay (RB), Ebb Tide Street (ES), Penguin Street (PS) and Tern Street (TS) sites in December 2003 and December 2005. Fauna data was fourth-root-transformed to down-weight the effects off very abundant species.

Within each site a main subset of the species cumulatively contributed to the 50% difference in abundance and number of species between studies (Table 3.13). At site CW *M. tenebrosus* had the largest change in abundance and *A. aureoradiata* the smallest (Table 3.13). *Aonides trifidus* and *Paraboccardia syrtis* were absent and *N. helmsi* and *H. filiformis* were present at site CH in 2005. At site RB *P. estuarinus*, *N. aestuariensis*, *Capitella sp.* and *Boccardia polybranchia* were present in 2005 but not in 2003. At site ES *A. crenata* and *S. cylindrifer* were in low abundance compared with 2005 and *M. liliana*, *A. stutchburyi* and *A. aureoradiata* were absent in 2003 but present in samples from 2005. At site PS in 2003 *A. stutchburyi* and *M. liliana* were absent but were present in 2005. Both *S. cylindrifer* and *A. crenata* were high in abundance at this site in 2005. In 2003 at site TS *N. helmsi*, *H. crassa* and the sipunculid worms were absent, but were present in samples from 2005 while, *P. syrtis* was absent in 2005 (Table 3.13).

Table 3.13: The mean change in abundance of invertebrate species (positive and negative) in the Causeway, Channel, Raupo Bay, Ebb Tide Street, Penguin Street and Tern Street sites between December 2003 and December 2005. Those species that cumulatively contribute to 50% of the variations between years are shown.

| Causeway | 2003 | 2005 | Channel | 2003 | 2005 |
|---------------------------------|----------|----------|---------------------------------|-------|-------|
| Species | Av.Abun. | Av.Abun. | Species | | |
| <i>Micrelenchus tenebrosus</i> | 37.3 | 18.11 | <i>Aonides trifidus</i> | 52.79 | 0 |
| <i>Heteromastus filiformis</i> | 3 | 20.81 | <i>Micrelenchus tenebrosus</i> | 5.57 | 38.47 |
| <i>Boccardia polybranchia</i> | 1 | 18.19 | <i>Notoacmea helmsi</i> | 0 | 29.12 |
| <i>Macomona liliana</i> | 10.95 | 27.57 | <i>Paraboccardia syrtis</i> | 20.32 | 0 |
| <i>Anthopleura aureoradiata</i> | 12.12 | 26.31 | <i>Heteromastus filiformis</i> | 0 | 19.21 |
| Raupo Bay | | | Ebb Tide Street | | |
| <i>Potamopyrgus estuarinus</i> | 0 | 16 | <i>Macomona liliana</i> | 0 | 17.78 |
| <i>Nicon aestuarienus</i> | 0 | 15.97 | <i>Austrovenus stutchburyi</i> | 0 | 14.14 |
| <i>Capitella sp.</i> | 0 | 14.28 | <i>Anthopleura aureoradiata</i> | 0 | 11.49 |
| <i>Boccardia polybranchia</i> | 0 | 12.88 | <i>Amphibola crenata</i> | 11.53 | 2 |
| | | | <i>Scoloplos cylindrifer</i> | 25.83 | 16.73 |
| Penguin Street | | | Tern Street | | |
| <i>Austrovenus stutchburyi</i> | 0 | 19.39 | <i>Notoacmea helmsi</i> | 0 | 14.14 |
| <i>Scoloplos cylindrifer</i> | 3.61 | 16.37 | <i>Helice crassa</i> | 0 | 10.77 |
| <i>Amphibola crenata</i> | 5.57 | 14.83 | Sipunculid worm | 0 | 10.1 |
| <i>Macomona liliana</i> | 0 | 8.72 | <i>Paraboccardia syrtis</i> | 8.72 | 0 |

3.2.5 Comparison between physico-chemical variables from 2003 and the present study

There were variations in physico-chemical variables and *U. lactuca* biomass between sites in 2003 and 2005. These are described below and summarised in Figure 3.15.

There were increases at all sites in dissolved oxygen, pore water and silt/clay between 2003 and 2005. Site CH had the only decrease in salinity between studies while organics had decreased at all sites by 2005. *U. lactuca* biomass increased at sites CW and CH and decreased at sites ES, PS and TS by 2005. Both studies showed differences in levels of physico-chemical variables between sites. Dissolved oxygen, salinity and organics were shown to have similar trends between sites in each study. There were no other clear trends shown between remaining variables.

In 2003, dissolved oxygen ranged from 3.9 at site RB to 9.16 at site TS compared to 6.2 at site RB to 12.3 mg/l⁻¹ at site CW in 2005. The greatest difference between years in levels were at site ES with 3.9 and the least at site PS with 1.1 mg/l⁻¹. Salinity ranged from 16.5 at site RB to 40 ppt at site CH in 2003 compared to 25.7 at site RB to 31.5 ppt at site TS in 2005. The greatest difference between years was at site CH with 10.5 and the lowest was at site TS with 1.53 ppt. In 2003, pore water ranged from 13.7 at site CW to 15.1 at site RB compared to 27.6 at site ES to 35.2% at site RB. The greatest difference between years in percentage of pore water was at site TS with 21.2 and the least at site PS with 11.6%. Organic matter ranged from 3.9 at site PS to 9.4% at site RB in 2003 compared to 1.6 at site PS to 4.2% at site CH in 2005. The greatest difference between years was at site RB with 5.2 and the least was at site TS with 1.8%. In 2003, percentage of silt/clay ranged from 6.4 at site ES to 16.8 at site RB compared to 8.3 at site PS to 67.7% at site RB in 2005. The greatest difference between years was at site RB with 50.9 and the lowest was at site PS with 0.3%. *U. lactuca* biomass ranged from 22.2 at site TS to 408 g per m² at site ES in 2003 compared to 0 at site TS to 293 g per m² at site CH in 2005. The greatest difference between years was at site ES with 351 and the smallest at site PS with 22 g per m².

Differences in physico-chemical variables between years were significant (ANOSIM, $R = 0.613$, $p = 0.002$; Fig 3.16). The only non significant variations between individual variables in 2003 and 2005 were in salinity and *U. lactuca* biomass (Table 3.14). Significant variations were between dissolved oxygen, pore water, organics and the silt/clay fraction (Table 3.14). There was no significant variation in temperature between studies (ANOVA, $F = 1.52$ (df=1, 54) $p = 0.247$).

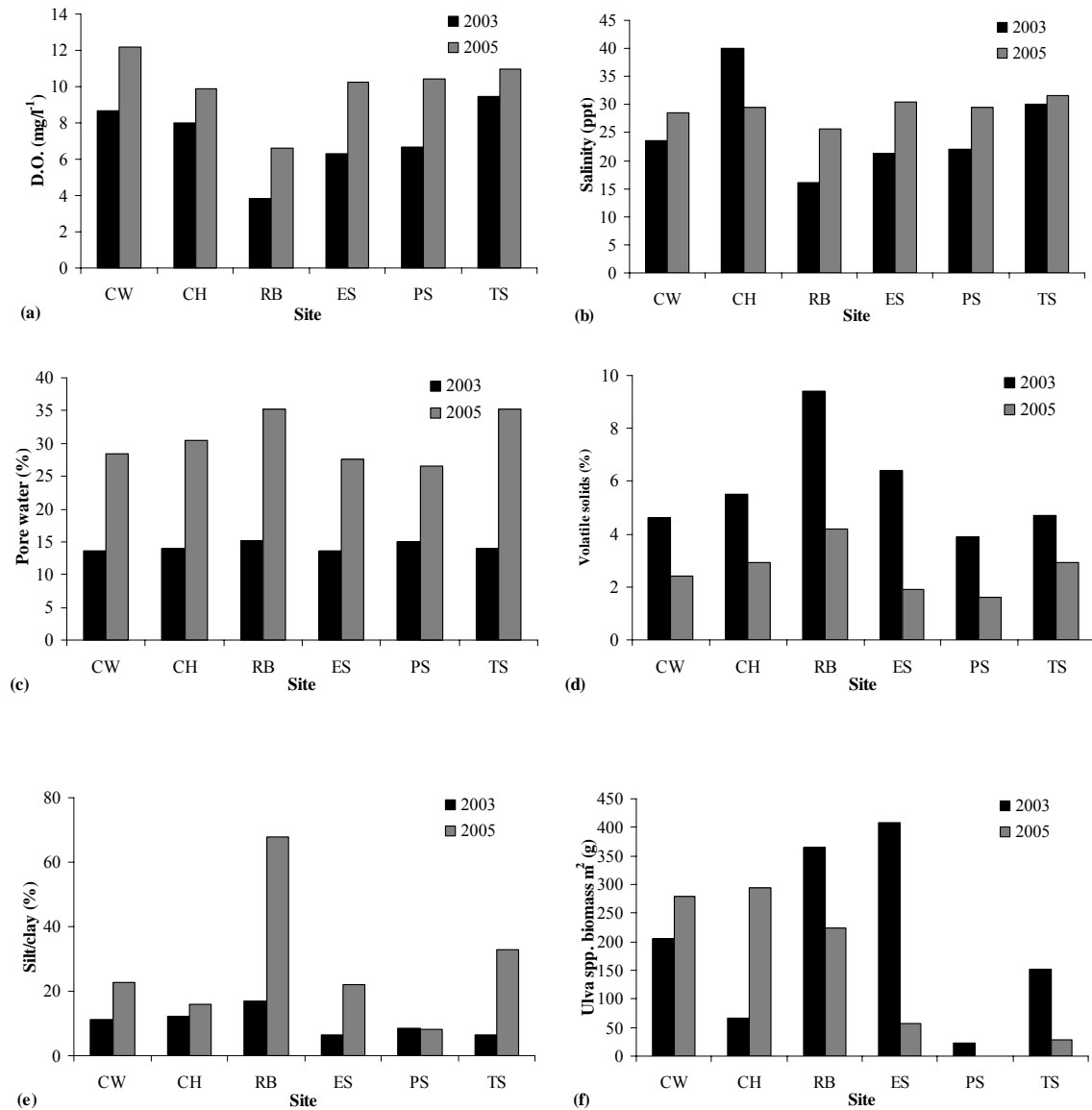


Figure 3.15: Physico-chemical variables (a) dissolved oxygen (mg/l⁻¹), (b) salinity (ppt), (c) pore water (%), (d) volatile solids (%) and (e) silt/Clay (%) and (f) *Ulva lactuca* biomass (g dry wt. m²) from sites CW, CH, RB, ES, TS, and PS in December 2003 and December 2005.

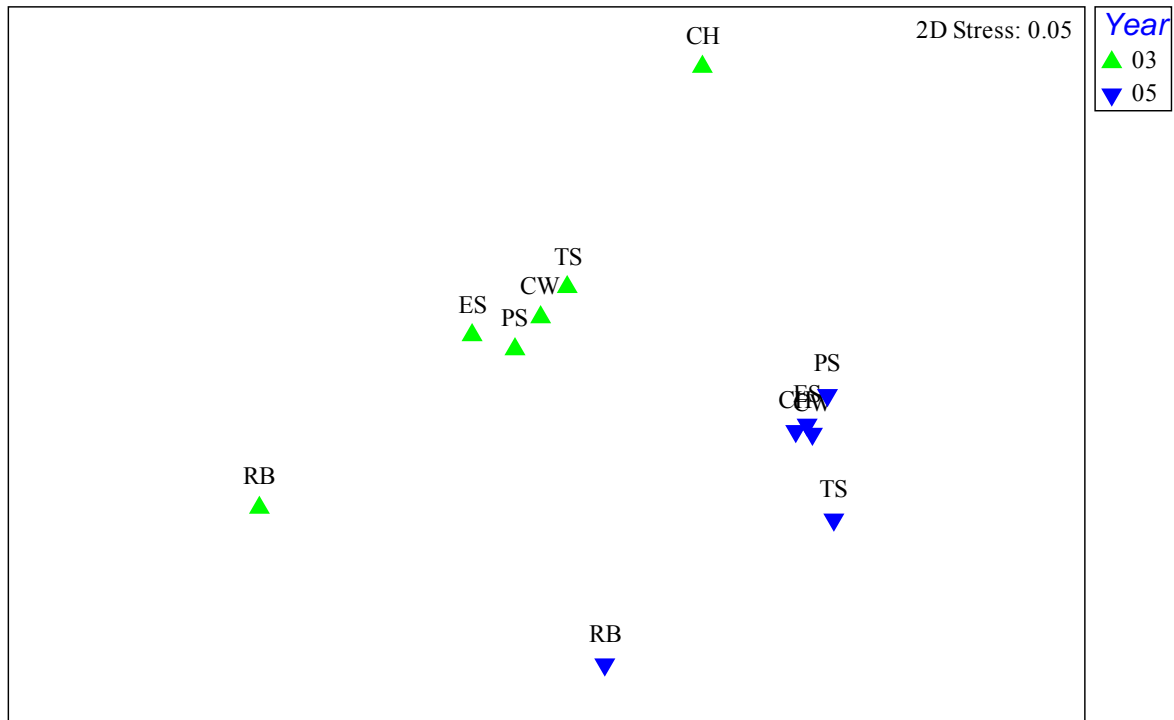


Figure 3.16: MDS ordination showing the similarity of physico-chemical variables (dissolved oxygen, temperature, salinity, pore water, volatile solids and silt/clay) and *Ulva lactuca* biomass between sites CW, CH, RB, ES, PS and TS in December 2003 and December 2005.

Table 3.14: Summary ANOVA results for the variation between physico-chemical variables between December 2003 and 2005 at sites CW, CH, RB, ES, PS and TS.

| Variable | F | p-value |
|-----------------------------|--------|---------|
| Dissolved oxygen | 5.991 | < 0.05 |
| Salinity | 1.478 | 0.252 |
| Pore water | 106.96 | < 0.001 |
| Volatile solids | 11.957 | < 0.01 |
| Silt/clay | 5.806 | < 0.05 |
| <i>Ulva lactuca</i> biomass | 0.556 | 0.473 |

3.2.6 *Ulva lactuca* zoospore settlement and growth

Six weeks after initiation of the experiment *U. lactuca* zoospores had attached and grown on tiles only at the Channel site (Fig. 3.17). These were identified as *U. lactuca* by comparing photographs of cell structure taken by Steffensen (1974) and photographs from the current study (Fig. 3.18). There was a significant difference between excluded and open treatments ANOVA $F = 18335.06$ ($df=1, 322$), $p < 0.001$.

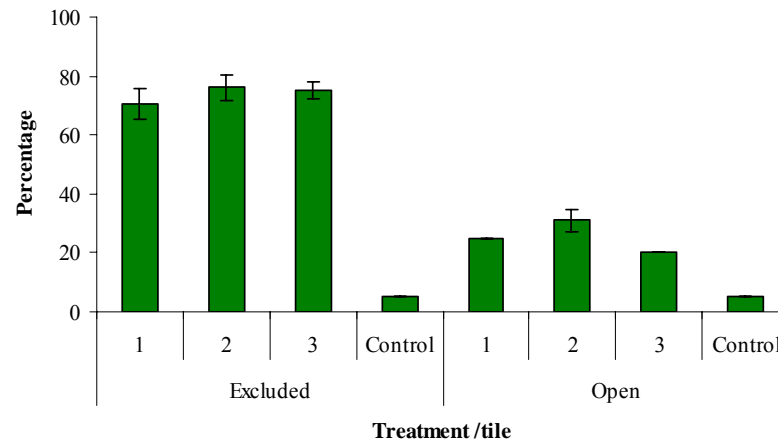


Figure 3.17: Mean percentage (\pm S.D.) cover of young *Ulva lactuca* plants at the Channel site on tiles with exclusion fences and tiles that were open.



Figure. 3.18: Cell structure and young plants of *Ulva lactuca* showing the stipe and developing thallus from tiles at the Channel site.

3.3 Discussion

Estuaries are a common coastal geological feature in New Zealand with approximately 300 being listed (McLay 1976). These soft bottom intertidal systems support a wide diversity of assemblages of benthic invertebrates from groups such as Decapoda, Gastropoda, Bivalvia and Polychaeta, and are dominated by mobile crustaceans and burrowing molluscs (Thrush *et al.* 1992; Ford *et al.* 1999; Hewitt *et al.* 2002; Thrush *et al.* 2003; Jones and Marsden 2005; Norkko *et al.* 2006). The most abundant groups have been recorded as Bivalvia and Polychaeta with species such as the bivalves *Austrovenus stutchburyi*, *Paphies australis* and *Macomona liliana*, and polychaetes *Heteromastus filiformis*, *Scolecopides* sp, and *Capitella* sp. being widely distributed throughout the countries intertidal systems. These species have been found in Whitford Estuary and embayment, North Island (Lohrer *et al.* 2004; Norkko *et al.* 2006), Manuka Harbour (Pridmore *et al.* 1990; Thrush *et al.* 1992), Mahurangi Harbour (Hewitt *et al.* 2002), Whitianga Harbour (Thrush *et al.* 2003), Okura Estuary (Norkko *et al.*

2002) and Papanui Inlet, Otago Peninsula (Ford *et al.* 1999). Other species commonly found in these systems include the amphipod *Paracorophium excavatum*, the bivalve *Arthritica bifurca* and the estuarine limpet *Notoacmea helmsi*. Species such as the horse mussel *Atrina zelandica* are more abundant in subtidal sandy and muddy soft sediments (Thrush *et al.* 1994; Thrush *et al.* 2003) of north east New Zealand such as Mahurangi Harbour compared to other systems in New Zealand (Hewitt *et al.* 2002). These systems support many other species from the groups mentioned above in varying abundances.

In the Avon-Heathcote Estuary, the dominant gastropod is *Amphibola crenata* and the dominant bivalve is *Austrovenus stutchburyi* (Stephenson 1981; Griffin and Thomson 1992). Polychaetes have been described in large abundances in this Estuary (Knox and Kilner 1973) and include the species *Capitella* sp. and *Heteromastus filiformis*. Many of these benthic invertebrates that inhabit estuarine systems exhibit seasonal cycles; densities increase from winter to summer (Ford *et al.* 1999; Hewitt *et al.* 2005). The distribution of benthic invertebrates is thought to be limited by gradients in physico-chemical gradients that are common to the Avon-Heathcote Estuary and similar systems throughout New Zealand (Thrush *et al.* 1994; Robertson *et al.* 2002; Thrush *et al.* 2003).

In this study differences in sediment physico-chemical variables between years of sampling most likely reflected temporal changes (Raffaelli *et al.* 1999). This was shown by similar trends in individual variables at each site in December; Raupo Bay best illustrates this where high silt/clay fraction coincided with higher percentages of pore water and lower levels of dissolved oxygen in each year (Fig. 3.16). Also, high biomass of *U. lactuca* coincided with high percentages of organics and low salinity in each study. There were no correlations between sediment variables and *U. lactuca* biomass from the general survey.

Salinity is thought to be the most important variable in limiting distribution of biota in estuarine systems (Day 1981; McLusky 1981). In this study salinity of over-lying water was not measured. However, salinity of sediment pore water was measured. It varied significantly between sites (refer page 48). Results of BIOENV analysis showed salinity of sediment pore water to be an important variable at the Channel site only (Table 3.9). At the remaining sites, various combinations of sediment temperature, silt/clay fraction, pore water and organics were shown to correlate best with assemblage patterns (Table 3.9).

Faunal biomass and species richness are affected by differences in sediment particle size; areas with high percentages of silt/clay have fewer species in low numbers compared to areas with high percentages of sand (Yates *et al.* 1993; Mannino and Montagna 1997; Cardoso *et al.* 2004b). Because of disaggregating of bound sediment particles for the purpose of analysis, determining the natural structuring effects on the benthic assemblages may not be

entirely accurate (Dernie *et al.* 2003). However, it was expected that percentages of silt/clay would be an important variable at McCormacks Bay and Raupo Bay in relation to the community assemblages. These sites had high percentages of silt/clay (Fig 3.7), low species richness (Table 3.2), and low numbers of individuals compared to other sites (Fig. 3.2) which were comparable to the studies by Yates *et al.* (1993) and Mannino and Montagna (1997). However, in the current general survey temperature and pore water were the most important variables at McCormacks Bay and Raupo Bay respectively (Table 3.9). Community composition and abundance has also been negatively affected by periodic anoxia (Diaz and Rosenberg 1995). The RDLs at McCormacks Bay and Raupo Bay were regularly observed to be at half a centimetre deep and levels of dissolved oxygen in sediment pore water were much lower than at any other site (Fig 3.7). This has been shown to negatively affect community assemblages by creating a hostile environment for benthic invertebrates resulting in decreased species richness and abundance (Theede *et al.* 1969; Modig and Olafsson 1998).

Results of this study showed variations in assemblages between and within sites over time (Fig. 3.5 and Fig. 3.6). These were correlated with the sediment variables: pore water temperature, pore water, organic matter and the percentage of silt/clay (Table 3.8 and Table 3.9). However, as estuarine invertebrates generally have a clumped distribution (Lewis and Taylor 1968), true relationships at the system scale between biological and physical components may not be as indicated by the local scale studies (Thrush *et al.* 1997; Edgar and Barrett 2002). This was illustrated when comparing variations in community assemblages at local scales between Bressington's study in 2003 and the current study.

Possible mechanistic explanations of the variations were indicated by results of the present preliminary study when comparing two quadrat sizes (Fig. 2.4). Sampling with larger quadrats greatly increased precision; greater number of species and individuals were found compared to smaller quadrats (Table 2.2) and these were found in fewer numbers of quadrats (Fig. 2.5). Bressington (2003) showed, in her preliminary study that precision of sampling was lower when using the 15 by 15 cm compared to the 25 by 25 cm quadrat. Regardless of this she chose the smaller quadrat, with three replicates at each site, for logistic reasons that included time, effort and accuracy when examining the samples. It is conceded that these reasons are often legitimate in ecological field studies especially when time and resources are factors (Schneider 1994; Quinn and Keough 2002). Changes in assemblages over years may then be partially attributed to differences in sample size and number of replicates used during each study (Hewitt *et al.* 1998). Therefore, it is important to standardise sampling and analysis protocols as this gives higher resolutions of comparison between studies over time (Raffaelli *et al.* 1999; Robertson *et al.* 2002).

U. lactuca biomass and cover remained low at all sites during the study (Fig. 3.7). The majority of species at each site showed a positive correlation with *U. lactuca* biomass. This is consistent with previous studies showing that in low biomass *Ulva* spp. reduces predation by providing a physical barrier to mudflat predators such as shore birds and fish (Hull 1987; Raffaelli *et al.* 1998; Lewis *et al.* 2003), increases species abundance because of protection from desiccation by the physical elements (Everett 1994) and provides a nursery environment for oviparous females (Ferreira *et al.* 2004) resulting in greater species diversity and abundance.

Condition of *A. stutchburyi* was higher at Penguin Street, Tern Street and, surprisingly also at McCormacks Bay. It is thought that high percentage of silt/clay, which is dominant at McCormacks Bay, has a negative effect on the physiology of bivalves (Norkko *et al.* 2006). At Penguin Street health dropped in November and December but not at Tern Street. High values of health have been associated with proximity to discharging oxidation pond effluents high in organic matter (Wong and Thompson 1992; Marsden 2004). None of these sites is in close proximity to the oxidation ponds (Fig. 3.1) or had high sediment organic matter (Fig. 3.7). It was assumed that column water salinity and phytoplankton availability at the Penguin Street and Tern Street sites would be higher than at other sites because of proximity to the Estuary mouth. Evidence has been found that reduced salinity and phytoplankton availability reduces condition and growth of *Austrovenus stutchburyi* (Marsden 2004). This would explain lower health indices at sites further from the Estuary mouth. High health indices at McCormacks Bay may be due to incoming tides bearing phytoplankton in adequate concentrations to the site and the supply of organic matter from decaying *U. lactuca*. Because of the low abundance of *Austrovenus stutchburyi* at site McCormacks Bay competition for food resources would not be a limiting factor in an individual's health. Variations in the seasonal condition of *Austrovenus stutchburyi* at sites such as Penguin Street (Fig. 3.10) may have been a result of spawning which generally occurs in summer (Larcombe 1971). The different seasonal patterns of condition in the Channel, Raupo Bay and Ebb Tide Street may be accounted for by spawning during different seasons (Norkko 2005). The higher number of individuals at the Channel site compared to McCormacks Bay may be related to sediment particle size. At the Channel site sand is the dominant sediment class whereas at McCormacks Bay silt/clay is dominant. Maximum densities of *Austrovenus stutchburyi* are found in sandy habitats such as at the Channel site (Thrush *et al.* 2003).

Hard substrate availability is thought to be a limiting factor for the distribution of *Ulva* spp. in soft-sediment habitats; substrate includes the shells of *Austrovenus stutchburyi*

and stones embedded in sediments (Morton and Miller 1973; Steffensen 1974). Grazing pressure is also a limiting factor to distribution and abundance of macroalgae species; germination of settled *Enteromorpha* spp. spores and growth of germlings were reduced by 93-99% in a laboratory study in 1995 (Lotze *et al.* 1999).

During the zoospore settlement and growth experiment the exclusion of grazing gastropods such as *Micrelenchus tenebrosus* and *Diloma* spp. led to a higher percent cover of *U. lactuca* (Fig. 3.17). Young plants were present on the edges of the control tiles but not on the sediment that had been glued to the upper flat surface. This indicated discrimination by the swimming and “searching” zoospores regarding suitability of substrates (Callow *et al.* 2000). It was suggested by Callow *et al.* (2000) that macroalgal zoospores were able to “sense” that hydrophobic surfaces were more favourable for settlement. They showed a positive correlation between the numbers of spores attached and increasing contact angle (hydrophobicity) (Callow *et al.* 2000). This is consistent with this study since *U. lactuca* was observed to be attached to hydrophobic cockleshells and no spores had settled on the surface of the control tiles. It was concluded that substrate is a limiting factor in *U. lactuca* zoospore settlement.

The absence of spore settlement and growth at Raupo Bay, Ebb Tide Street, Penguin Street and Tern Street indicates that zoospores were unable to disperse to these areas from seed sites such as McCormacks Bay, the Causeway and Channel sites. However, attached plants were observed at these sites during the current research. The mechanistic explanation for this may be that plants, or parts of their reproductive thallus were detached and drifted to sites such as Raupo Bay and Ebb Tide Street, and then were buried, and remained viable over winter in the sediments. Later, in spring, such detached plants may become exposed and produce spores that attach to the sparsely available substrates such as shells and small pebbles as reported by Kamermans *et al.* (1998).

Zoospores were not capable of dispersing from the western side of the Estuary to sites on the eastern side. A likely explanation for this would be the hydrodynamics and currents in the Estuary acting against zoospore dispersal to the eastern sites. Also, although *Ulva* spp. zoospores were determined to have a wide dispersal shadow (Littler and Littler 1980), to remain viable for greater than six days after spore release and to have slow sinking rates compared to other algal species because of their small size (9.91 µm) (Hoffmann and Camus 1989), it is unlikely that the *U. lactuca* zoospores’ dispersal shadow would be as far as reaching from one side of the Estuary to the other. This is because time to remain suspended in the Avon-Heathcote Estuary’s column water would be limited because of the tidal cycle and the amount of water exchange per tide (refer to Section 1.2). The typical dispersal

patterns observed from winter to summer at the sites during the current research indicated that the main seed sites were McCormacks Bay, Causeway and Channel Site.

In conclusion, the present study showed variations in assemblages between seasons which were driven mainly by temperature (Table 3.9). However, sediment particle size is thought to be an important physical variable in structuring benthic assemblages (Yates *et al.* 1993). There is evidence that benthic invertebrate assemblages and abundance have changed between 2003 and 2005. However, the high degree of change is likely to result from different sampling protocols. This makes generalisations from the comparisons difficult (Raffaelli *et al.* 1999; Robertson *et al.* 2002). It is therefore important to implement standards for sampling protocols within the Estuary such as those used by this study and Maclaren (2005) in which sample size and replicates were the same. This allows for a high resolution of comparability (Hewitt *et al.* 1998). It was also concluded, that focusing control on *U. lactuca* spore sites such as McCormacks Bay, Causeway and the Channel site would reduce the overall potential biomass within the Estuary.

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(Norkko and Bonsdorff 1996b; Cardoso *et al.* 2004a)

Chapter 4

Effects of mechanically removing *Ulva lactuca* L. mats on benthic invertebrates and physico-chemical variables

4.1 Introduction

Globally, estuarine ecosystems are subjected to anthropogenic eutrophication especially in high density industrial and populated areas (Cloern 2001; Auffrey *et al.* 2004; Cardoso *et al.* 2004b; Zhou *et al.* 2004). Increased eutrophication results from high inputs of nutrients and excessive production of organic matter. This results in poor water quality and clarity or depleted dissolved oxygen and can trigger serious biological changes such as an overall increase in primary production and contamination (Kiddon *et al.* 2003; Rosales-Hoz *et al.* 2003; Cardoso *et al.* 2004a; Zhou *et al.* 2004). This is chiefly caused by discharge of effluent, agriculture, industry (Dauer and Conner 1980; Rosales-Hoz *et al.* 2003; Korpinen *et al.* 2004; Lee *et al.* 2004; Norkko *et al.* 2006), aquaculture (Auffrey *et al.* 2004) and changes in hydrology (Knox and Kilner 1973; Soulsby *et al.* 1982; Raffaelli *et al.* 1998). A direct consequence of high nutrient loading is increase of naturally occurring macroalgal blooms and mats (Soulsby *et al.* 1982; Everett 1991; Fletcher 1996; Norkko *et al.* 2000; Franz and Friedman 2002; Cardoso *et al.* 2004b). These affect water quality, community structure (Raffaelli *et al.* 1991; Thiel and Watling 1998), infaunal burrowing behaviour (Norkko and Bonsdorff 1996b; Norkko and Bonsdorff 1996a; Osterling and Pihl 2001; Auffrey *et al.* 2004) and sediment chemistry (Olafsson 1988; Bolam *et al.* 2000). The result is a complex mix of direct and indirect scale-dependent interactions (Hull 1987; Raffaelli *et al.* 1998) (Fig. 4.1) which, have become an important global marine problem (Ford *et al.* 1999; Auffrey *et al.* 2004; Cardoso *et al.* 2004b; Jones and Pinn 2006).

Effects of macroalgae on benthic fauna

Difficulties arise in making generalisations about effects of macroalgal mats on soft sediment invertebrate assemblages. This is because differences between studies occur in experimental variables such as algal species, biomass, location of study sites, type of system

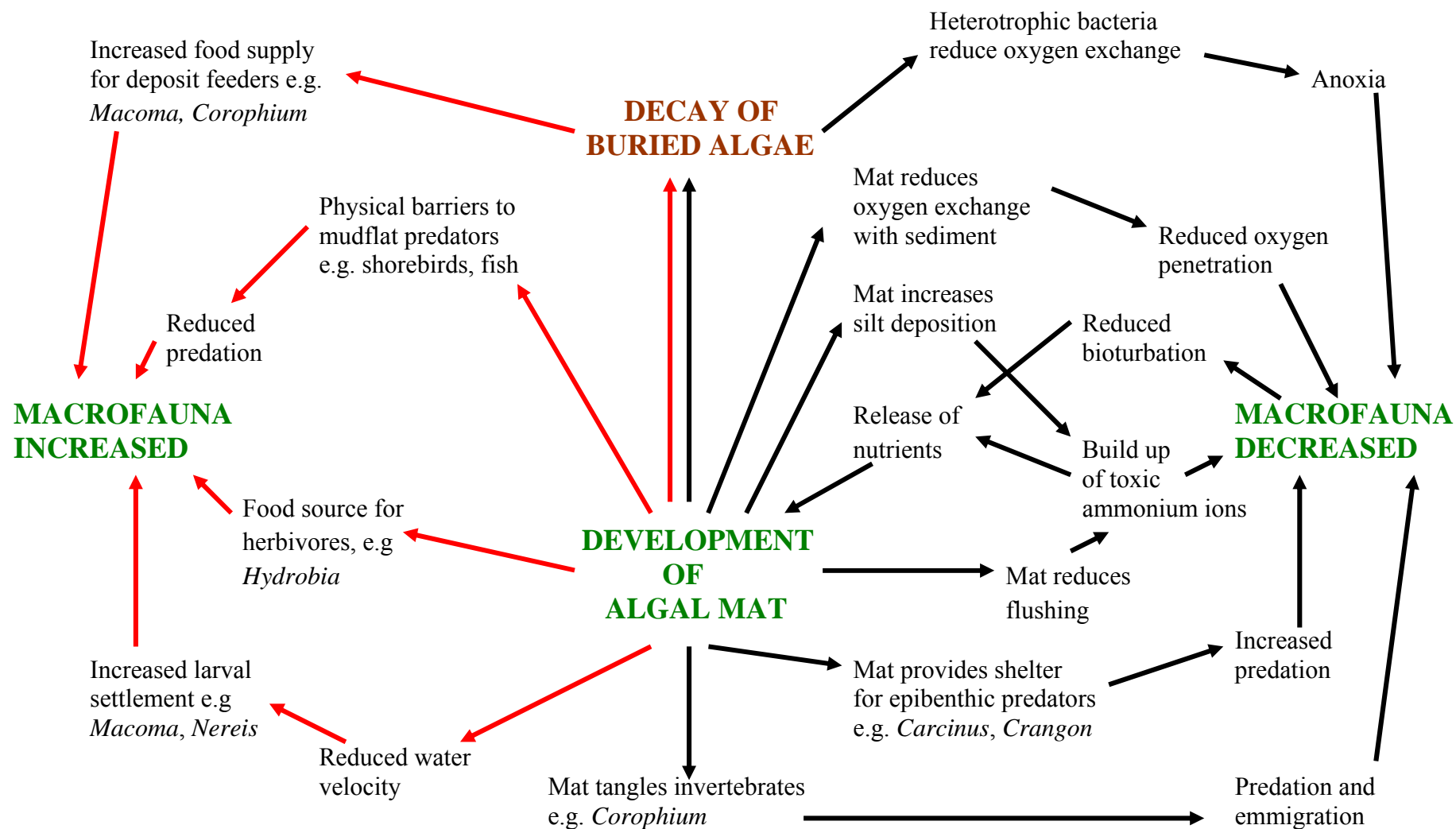


Figure 4.1: Effects of macroalgal mats on sediment physico-chemistry, invertebrates and predators. Red arrows = positive and black arrows = negative effects on invertebrates (modified from Hull 1987 and Raffaelli *et al.* 1998).

and abiotic factors (Everett 1994). However, it is generally accepted that macroalgae have a strong influence on the structure of benthic faunal assemblages (Nicholls *et al.* 1981; Hull 1987; Everett 1994; Norkko and Bonsdorff 1996b; Thiel and Watling 1998; Bolam *et al.* 2000; Lopes *et al.* 2000; Raffaelli 2000; Franz and Friedman 2002; Auffrey *et al.* 2004; Cardoso *et al.* 2004b; Jones and Pinn 2006). As macroalgal mats develop, faunal diversity and abundance increases (Fig. 4.1). This is followed by rapid faunal declines as mats increase in density (Lopes *et al.* 2000; Jones and Pinn 2006). Initial increase in fauna abundance has been attributed to reduced water velocity caused by development of macroalgal mats facilitating increased larval settlement and protection from predation especially for oviparous females of mobile species (Hull 1987; Escartin and Aubrey 1995; Cardoso *et al.* 2004b; Ferreira *et al.* 2004) (Fig. 4.1).

Experiments involving removal of macroalgae showed that laminar forms such as *Ulva* spp. had mixed effects on benthic fauna (Raffaelli *et al.* 1998; Cardoso *et al.* 2004b). A study in Bodega Harbour, California showed that densities of sedentary species such as the bivalve *Macoma balthica* and tube dwelling polychaetes were much lower in algal covered areas, and mobile sediment-interface feeding species had greater densities compared to clear areas (Everett 1994). *Ulva* spp. removal resulted in significant increases in density and species richness of copepods in Jamaica Bay, New York (Franz and Friedman 2002) and sedentary benthic infauna were less capable than mobile epifauna in dispersing to cleared sites in Clonakilty Estuary, Southern Ireland (Lewis *et al.* 2003).

Dominance shifts from epifaunal amphipods to burrowing detritivores such as oligochaetes and the polychaete *Capitella* spp. under macroalgal mats are common occurrences (Norkko and Bonsdorff 1996b; Thiel and Watling 1998; Raffaelli 1999; Lopes *et al.* 2000; Andersen *et al.* 2005). Artificially implanted algae, *Enteromorpha prolifera*, in the Firth of Forth, Scotland caused a significant increase of macrofaunal diversity including *Capitella capitata*, oligochaetes and *Gammarus* spp. while bivalves *Cerastoderma edule* and *Macoma balthica* showed no difference in abundance. These events coincided with significant decrease in the abundance of the polychaete *Pygospio elegans* (Bolam *et al.* 2000).

Macroalgal mats also affect burrowing behaviour of benthic infauna by causing upward migration; burial depth of the soft-shelled clam *Mya arenaria* (L.) in Bay of Fundy, Canada, was found to be shallower for individuals under macroalgal mats than those in clear areas (Auffrey *et al.* 2004). Tallqvist (2001) reported similar results from laboratory studies using *Macoma balthica* (L.). She suggested that negative influences on the burial depth of *M. balthica* may make it susceptible to predators at the sediment surface.

Finally, loss of the amphipod *Corophium volutator*, considered the most important invertebrate food source for epibenthic crustaceans, fish and shorebirds, coincided with blooms of macroalgal mats in Ythan Estuary, Scotland (Raffaelli 1999).

Effects of macroalgae on sediment physico-chemistry

Development of macroalgal mats is thought to alter hydrodynamics of estuarine systems by reducing their flushing potential (Hull 1987) and decreasing water flow around and through mats (Escartin and Aubrey 1995). Extents of these effects are dependent on velocities of wave, wind and tidal action combined with system depth and algal mat density (Escartin and Aubrey 1995). Reduced water velocity accounts for increases in silt/clay deposition in macroalgal areas altering sediment particle composition (Hull 1987; Escartin and Aubrey 1995) (Fig 4.1). Heavy metals such as zinc, copper and aluminium occur naturally in these sediments and, when accumulated or leached into the system, contribute to long-term contamination of the area (Oreja and Salinas 2003; Rosales-Hoz *et al.* 2003). Percent pore water and organics from decaying macroalgae significantly increase in underlying sediments which become anoxic accumulating toxic hydrogen sulphide (Raffaelli *et al.* 1998; Bolam and Fernandes 2002; Wetzel *et al.* 2002). This is because the mats and heterotrophic bacteria, which respire aerobically, reduce oxygen exchange at the sediment/water interface (Hull 1987) (Fig. 4.1). Under these conditions it is thought that remineralisation of nutrients by heterotrophic bacteria takes place within the sediments, which act as a source/sink promoting further macroalgal growth (Astill and Lavery 2001; Sundback *et al.* 2003). The end result of the development and decay of macroalgal mats is the generation of a hostile physico-chemical environment in the underlying sediments that is detrimental to the majority of benthic fauna (Soulsby *et al.* 1982; Raffaelli *et al.* 1991; Norkko and Bonsdorff 1996a; Raffaelli *et al.* 1998; Raffaelli 2000; Bressington 2003).

Macroalgal blooms in the Avon-Heathcote Estuary

Ulva lactuca L. has been increasing in the Avon-Heathcote Estuary since 1929 and blooms have been periodically reported over the past 60 years (Knox and Kilner 1973; Robertson *et al.* 2002). Blooms have been attributed to high concentrations of nitrogen and phosphorus in treated effluents released by the Christchurch Waste-water Treatment Plant (Knox and Kilner 1973). Previous studies on macroalgae in the Estuary have focused on abundance and spatial distribution (Knox and Kilner 1973; Steffensen 1974), effect of current velocity on thallus detachment (Hawes and Smith 1995), effects of macroalgal mats on

benthic fauna (Bressington 2003), and investigation of effects of benthic macroalgae on the cockle *Austrovenus stutchburyi* (Maclaren 2005).

Aims

The aims of this study were to determine effects of mechanically removing mat-forming macroalgal *Ulva lactuca* L. on invertebrate species abundance and sediment physico-chemical variables at two sites in Avon-Heathcote Estuary. The effects were examined through univariate and multivariate analyses (Chapter 2, section 2.4). It was expected that epifaunal species would initially decrease in abundance at each site. Over time it was thought that condition indices (CI) of the infauna bivalve *Austrovenus stutchburyi* would improve in areas of removal more than in undisturbed plots. It was expected that recruitment by immigration from surrounding areas would rapidly repopulate cleared plots and become similar in faunal composition to an undisturbed habitat. Disturbance to and exposure of sediments by removal of macroalgal mats were predicted to promote changes in sediment chemistry and particle composition.

4.2 Methodology

Two contrasting sites were established on intertidal mud and sandflats covered with *U. lactuca* in Avon-Heathcote Estuary, South Island, New Zealand in 2005; McCormacks Bay (MB), an enclosed embayment dominated by mudflats, in February, and Channel (CH), an open exposed sandflat, in March (Fig. 1.3). At each site twelve 8 x 2 m permanent plots were marked at the corners with wooden stakes (Fig. 2.3). Plots were randomly assigned to one of two treatments: unmanipulated (control), or *U. lactuca* removal (R) (Table 4.1). These were sampled one day pre, one day post and 46 days after removal of *U. lactuca* at similar times and tidal levels (Table 4.2). This was to reduce effects of diurnal fluctuations. Benthic invertebrates (including CI for *Austrovenus stutchburyi*) and physico-chemical variables were sampled and calculated following the methodology described in Chapter 2.

At both sites additional sediment samples to measure heavy metals, nitrogen and phosphorus were taken using 5 cm diameter, 5 cm depth (volume ~ 98.12 cm³) corers. Samples were placed in sterile plastic air tight containers supplied by the Christchurch City Council City Water and Waste Unit Laboratory. To reduce the cost of analysis samples were combined in treatments from left to right at each site. This resulted in three control and three removal samples from each site and sampling time. The Water and Waste Unit Laboratory measured concentrations (mg/kg) of copper (Cu), chromium (Cr), nickel (Ni), zinc (Zn), cadmium (Cd) and lead (Pb) using *APHA 3111B* – Atomic Absorption Spectroscopy. Arsenic

(As) was calculated using *APHA 3125 B* – ICP-MS, and total phosphorus (TP) was calculated using *ALPHA 20th Edn 4500N C* by R. J. Hill Laboratories. Total nitrogen (TN) was calculated using ICP-MS US EPA 200.2 by the Cawthron Institute. Because of combining samples data analysis on heavy metals, nitrogen and phosphorus were conducted separately from other physico-chemical variables.

Table 4.1: Layout of unmanipulated (C = control) and manipulated (R = removal) plots at the McCormacks Bay and Channel sites. Treatments were assigned at random.

| McCormacks Bay | | | | | |
|----------------|--------|--------|--------|--------|--------|
| MB1 R | MB2 C | MB3 R | MB4 C | MB5 C | MB6 R |
| MB7 C | MB8 R | MB9 R | MB10 C | MB11 R | MB12 C |
| Channel | | | | | |
| CH6 C | CH5 R | CH4 C | CH3 R | CH2 R | CH1 C |
| CH12 R | CH11 C | CH10 R | CH9 C | CH8 C | CH7 R |

Table 4.2: Sampling dates, times and tidal height in the Avon-Heathcote Estuary for the removal experiments at the McCormacks Bay and Channel sites in 2005.

| McCormacks Bay | | | |
|----------------|------------|------------|--------------|
| Sample | Month/date | Time | Low tide (m) |
| Pre-removal | April 6th | 9.18 a.m. | 0.3 |
| Removal | April 7th | 10.11 a.m. | 0.2 |
| Post-removal | April 8th | 11.03 a.m. | 0.2 |
| Final sample | May 24th | 11.36 a.m. | 0.4 |
| Channel | | | |
| Sample | Month/date | Time | Low tide (m) |
| Pre-removal | May 5th | 9.00 a.m. | 0.3 |
| Removal | May 6th | 9.50 a.m. | 0.2 |
| Post-removal | May 7th | 10.40 a.m. | 0.3 |
| Final sample | June 22nd | 11.11 a.m. | 0.4 |

4.3 Results

4.3.1 Testing similarity between sites

Community composition

Mean number of species were greater at site CH than at site MB but the differences were not significant (ANOVA, $p_{(df=1, F=1.22)} = 0.30$; Fig.4.2). There was a significant difference (ANOSIM, $R=0.998$, $p<0.001$) in community composition and abundance of individuals between sites (Table 4.3), as illustrated by Figure 4.3. Species contributing to these differences were *Zeacumantus subcarinatus*, *Micrelenchus tenebrosus*, *Zeacumantus lutulentus*, *Austrovenus stutchburyi*, *Macomona liliana* and *Amphibola crenata* (Table 4.3). There was also a significant difference in CI of *A. stutchburyi* between sites (ANOVA, $p_{(df=1, F=80.32)} <0.001$). Individuals of this species also differed in size and density between the two communities. At site MB, they ranged from 10.31 to 42.94 mm with a mean of $26.23 (\pm S.D. 7.37)$ mm. The mean density was $2.66 (\pm S.D. 1.60)$ per 0.0625 m^2 . At site CH, the range was 12.38 to 48.20 mm with a mean of $35.40 (\pm S.D. 6.97)$ mm, and mean density of $57.92 (\pm S.D. 14.68)$ per 0.0625 m^2 .

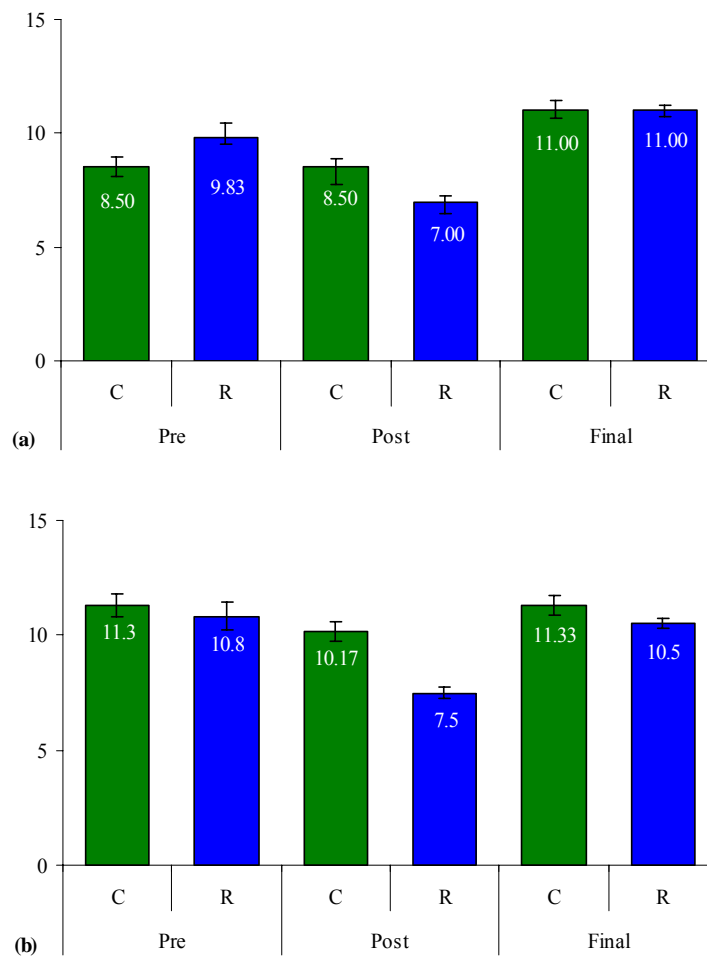


Figure 4.2: Mean ($\pm S.E.$) number of species from control (C) and removal (R) treatments for each sampling time at (a) MB and (b) CH. The mean number for each treatment is embedded in each bar.

Table 4.3: Mean number (0.0625m^2 , $\pm\text{S.D.}$) of each species found at sites MB and CH from all sampling times during the removal study. The five rarest species are in bold.

| Species | McCormacks Bay | Channel |
|--|-------------------|-------------------|
| <i>Helice crassa</i> | 0.83(1.45) | 3.22(3.55) |
| <i>Haliscarcinus whitei</i> | 0 | 0.31(0.40) |
| <i>Xymene plebeius</i> | 0 | 0.39(0.33) |
| <i>Cominella maculosa</i> | 0.06(0.24) | 0 |
| <i>Cominella glandiformis</i> | 1.06(0.67) | 0.68(0.47) |
| <i>Micrelenchus tenebrosus</i> | 1.26(1.10) | 62.73(43.09) |
| <i>Diloma subrostrata</i> | 0.16(0.28) | 2.93(1.59) |
| <i>Diloma nigerrima</i> | 0.16(0.29) | 0.02(0.09) |
| <i>Amphibola crenata</i> | 2.94(2.10) | 0.01(0.04) |
| <i>Notoacmea helmsi</i> | 0.71(0.75) | 8.51(5.67) |
| <i>Cellana radians</i> | 0 | 0.24(0.50) |
| <i>Zeacumantus subcarinatus</i> | 18.39(22.13) | 0 |
| <i>Zeacumantus lutulentus</i> | 8.03(10.49) | 0 |
| <i>Potamopyrgus estuarinus</i> | 0.01(0.04) | 0 |
| <i>Austrovenus stutchburyi</i> | 2.22(1.47) | 60.43(14.24) |
| <i>Macomona liliana</i> | 0.01(0.04) | 6.67(3.25) |
| <i>Arthritica bifurca</i> | 2.57(3.33) | 2.76(2.46) |
| <i>Mytilus edulis galloprovincialis</i> | 0 | 0.06(0.16) |
| <i>Paphies australis</i> | 0.01(0.08) | 0 |
| <i>Anthopleura aureoradiata</i> | 1.63(1.50) | 15.31(15.31) |
| <i>Melita awa</i> | 0.03(0.13) | 0 |

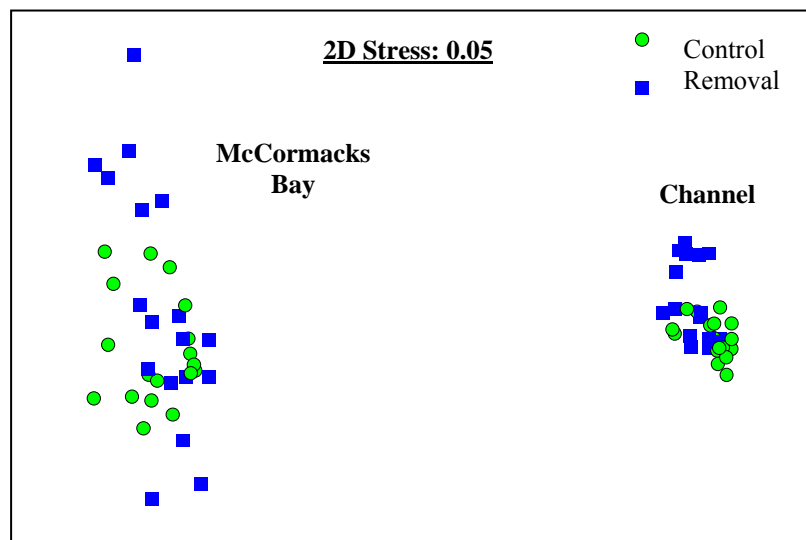


Figure 4.3: MDS ordination showing differences between community composition at sites MB and CH. Each symbol represents either a control or removal plot ($n = 4$ per plot) at a particular monitoring time from all sampling periods. The 2D stress of 0.05 indicates a high level of accuracy of the graphical representation by MDS.

Ulva lactuca biomass

There was no significant difference in *U. lactuca* biomass between sites prior to removal (ANOVA, $P_{(df=1, F=1.42)} = 0.25$; Fig. 4.4). The dry weight (g) ranged from 0.72 to 11.45 at site MB with a mean of $3.37(\pm\text{S.D. } 2.76)$ (Fig. 4.4a). At site CH, dry weight (g) ranged from 0.87 to 6.17 with a mean of $2.25 (\pm\text{S.D. } 1.72)$ (Fig. 4.4b). From visual estimates percent cover appeared to be uniform at each site.

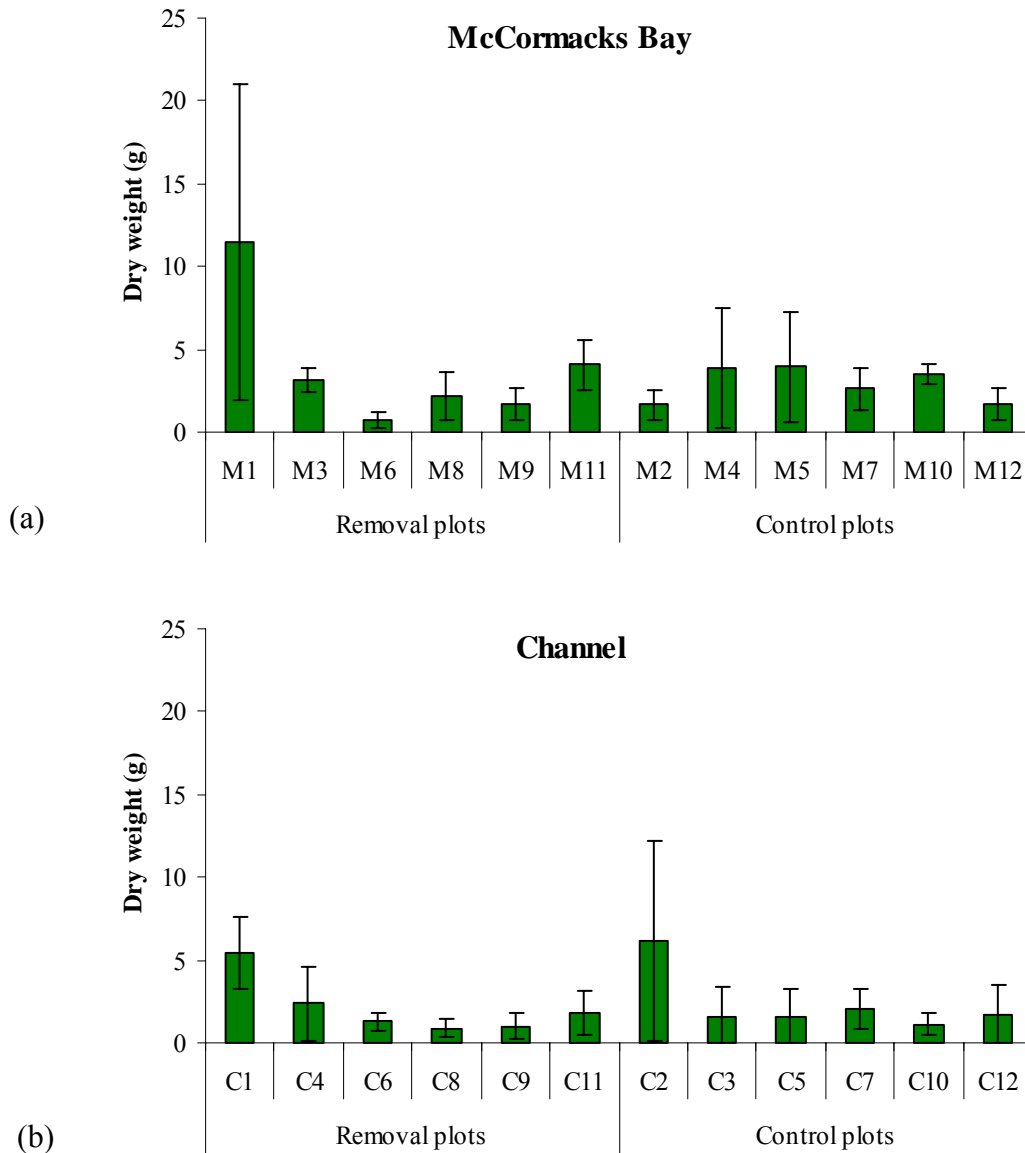


Figure 4.4: Mean ($\pm\text{S.D.}$) dry weight (g) of *Ulva lactuca* at sites (a) McCormacks Bay and (b) Channel prior to the removal experiment.

Environmental gradients

Environmental gradients can occur at a range of different temporal and spatial scales. Regular spacing of plots (Fig 2.3) may coincide with these gradients. Thus any inferences to the whole population of possible sampling units could be biased. Therefore it is important to analyse environmental data such as salinity or sediment composition for unknown gradients within the study site (Quinn and Keough 2002). PCA ordinations (Chapter 2.4) were conducted on the salinity, dissolved oxygen, temperature, pore water, organic matter and silt/clay fraction data collected from all plots at each site. Pairwise ANOSIMs (Chapter 2.4) were run on the same data in three blocks containing four plots from left to right at each site.

PCA ordinations (Fig. 4.5) showed no gradients in physico-chemical variables as shown by the pattern of plots compared to their layout at each site (Table 4.1). There were no significant variations between blocks at sites MB and CH; ANOSIMs (Global $R=0.204$, $p=0.05$ and Global $R=-0.093$, $p=0.75$) respectively. The Global R values showed the blocks within each site to be highly similar. Values being closer to zero = similarity and closer to one = dissimilarity.

Comparisons of similarity in physico-chemical variables and community composition between sites were made. This was to determine whether treatments should be compared within sites or between sites.

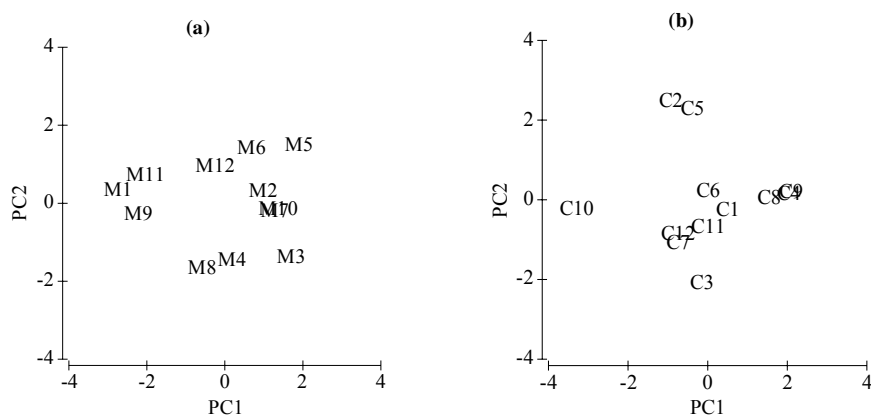


Figure 4.5: Two-dimensional PCA ordination of physico-chemical variables (salinity, dissolved oxygen, temperature, pore water, volatile solids, and silt / clay fraction) $n=4$ per sample for each of the 12 plots prior to removal at (a) McCormacks Bay; PC1 and PC2 together account for 64.4%, and (b) the Channel site; PC1 and PC2 together account for 62.9% of the total sample variability.

Physico-chemical variables

Combined physico-chemical variables (excluding heavy metals, nitrogen and phosphorus) were significantly different between sites (ANOSIM, $R=0.812$, $p<0.001$; Fig. 4.6). Individually, all variables were significantly different between the sites (Table 4.4). Silt/clay, sediment pore water and dissolved oxygen showed the most pronounced differences. The most similar variables between sites were sediment salinity and temperature (Table 4.4).

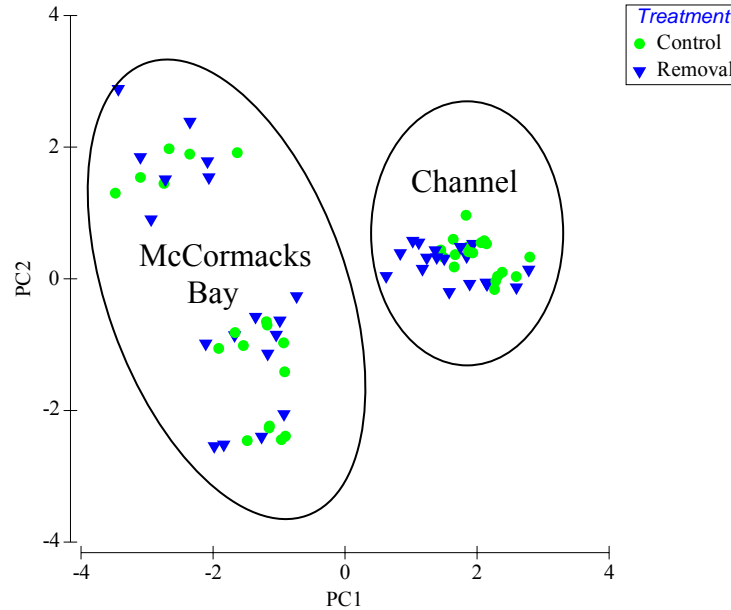


Figure 4.6: PCA ordination showing physico-chemical variables in control and removal treatments at sites MB and CH. Each symbol represents a plot (mean of $n = 4$ per plot) at a particular monitoring time from all sampling periods. PC1 and PC2 together account for 88.5% of the total sample variability.

Table 4.4: Mean physico-chemical values (\pm S.D.) from all sampling times and summary of ANOVAs ($\alpha=0.05$) comparing the differences between the pre-removal physico-chemical samples from sites MB and CH.

| | MB | CH | | |
|---|--------------|--------------|---------|---------|
| Variable | Avg | Avg | F | p-value |
| Dissolved Oxygen (mg l^{-1}) | 04.14 (0.01) | 09.66 (0.02) | 9447.61 | <0.001 |
| Silt/clay (%) | 74.27 (4.33) | 16.93 (5.58) | 794.09 | <0.001 |
| Pore water (%) | 31.45 (1.41) | 25.16 (1.35) | 42.37 | <0.001 |
| Organic matter (%) | 03.26 (0.30) | 02.38 (0.26) | 37.00 | <0.001 |
| Salinity (ppt) | 31.91 (1.01) | 31.40 (0.20) | 12.72 | <0.001 |
| Temp ($^{\circ}\text{C}$) | 15.71 (0.04) | 16.43 (0.04) | 4827.82 | <0.001 |

Heavy metals, nitrogen and phosphorus

Concentrations of the heavy metals (excluding cadmium), nitrogen and phosphorus varied between sites (Appendix 3) and were significantly dissimilar (ANOSIM, $R=1$, $p<0.002$; Fig. 4.7) with the most significant difference in levels of total nitrogen (ANOVA, $P_{(df=1, F=369.87)} <0.001$; Fig. 4.8). Variations in concentrations are described below and summarised in Figure 4.8.

Cadmium concentrations were 2 mg/kg at each site, time and treatment and therefore excluded from any data analysis. At site MB, copper, zinc, lead, total nitrogen and total phosphorus were at higher levels than at site CH. At site CH, chromium, nickel and arsenic were at higher concentrations compared to site MB. Copper levels ranged from 8.1 to 10.4 mg/kg at site MB, and 4.5 to 5.5 mg/kg at site CH. At site MB, zinc ranged from 61.5 to 81.5 mg/kg and 55 to 60 mg/kg at site CH. Lead ranged from 17 to 26 mg/kg at site MB and 11 to 16 mg/kg at site CH. Total nitrogen and total phosphorus ranged from 730 to 1100 mg/kg, and 432 to 524 mg/kg at site MB respectively. At site CH, total nitrogen and total phosphorus ranged from 410 to 580 mg/kg, and 346 to 470 mg/kg respectively. Chromium ranged from 18 to 21 mg/kg at site CH and 12.5 to 18 mg/kg at site MB. At site CH, nickel ranged from 11 to 14 mg/kg and from 9.2 to 14 mg/kg at site MB. Arsenic ranged from 2 to 3 mg/kg at site CH and 2.4 to 3.5 mg/kg at site MB.

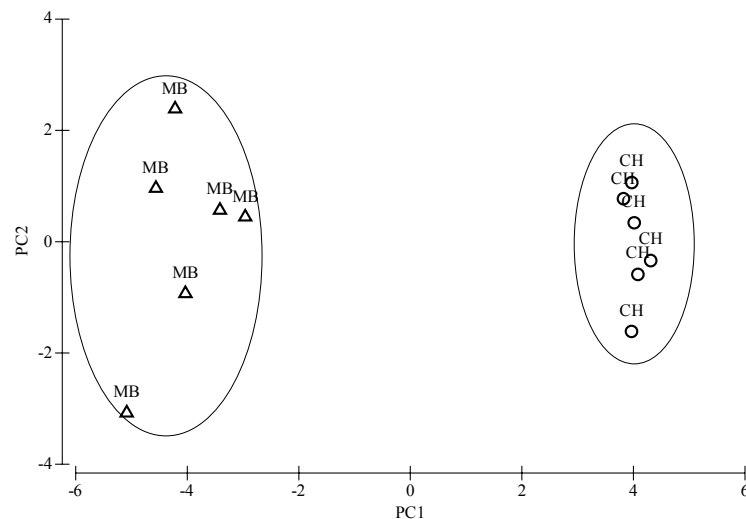


Figure 4.7: Two-dimensional PCA ordination of square-root-transformed and normalised data of heavy metals in the sediment (chromium, copper, lead, nickel and zinc, and total nitrogen and phosphorus) for McCormacks Bay and the Channel site from the pre-removal sampling. PC1 and PC2 together account for 83.7% of the total sample variability.

Because of the differences in community composition, physico-chemical variables, heavy metals, nitrogen and phosphorus, treatments were compared separately within sites.

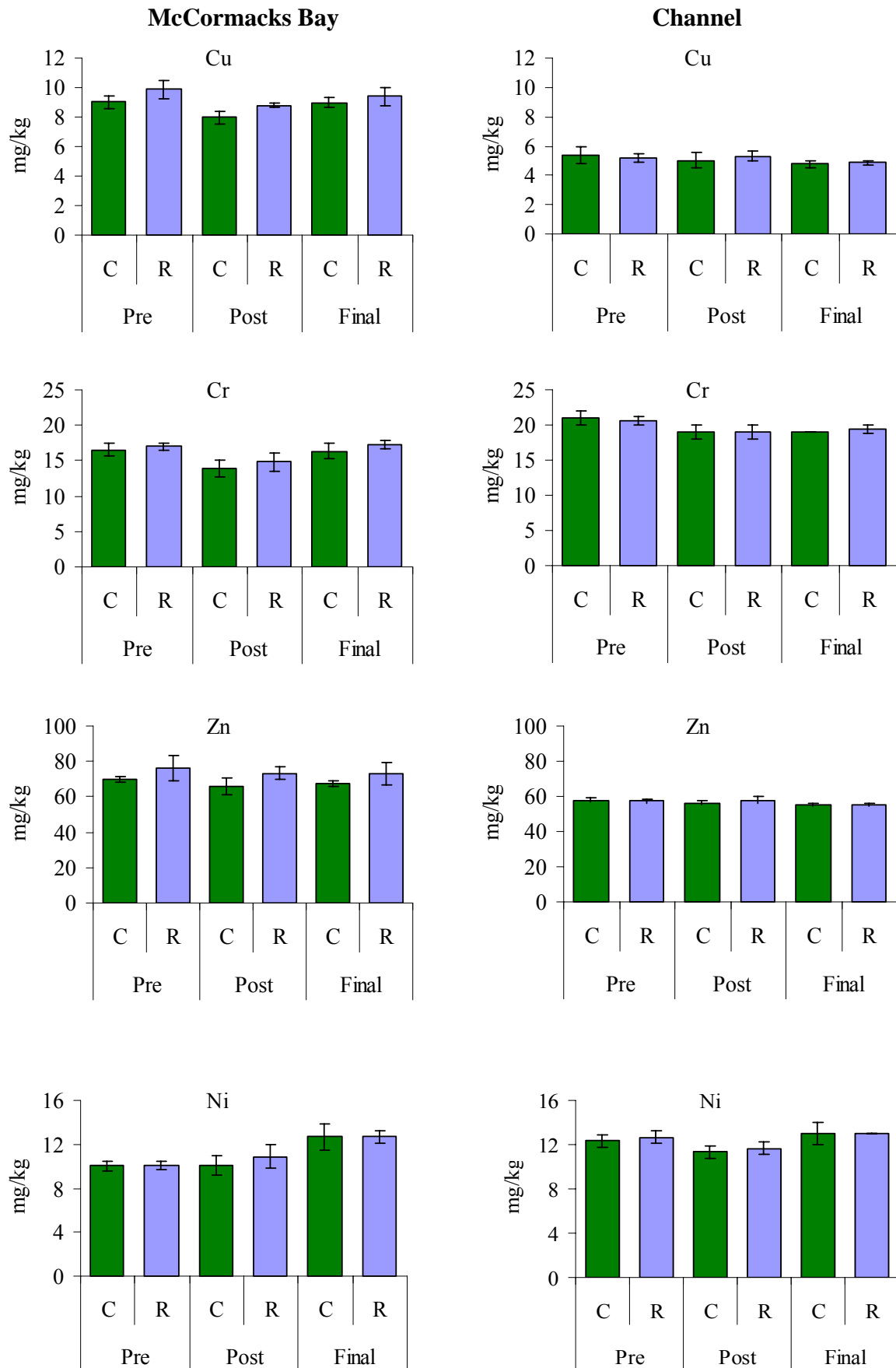


Figure 4.8: Mean (\pm S.D.) concentrations of copper, chromium, zinc, nickel, lead, arsenic, nitrogen and phosphorus from control (C) and removal (R) treatments for each sampling time at McCormacks Bay and the Channel site.

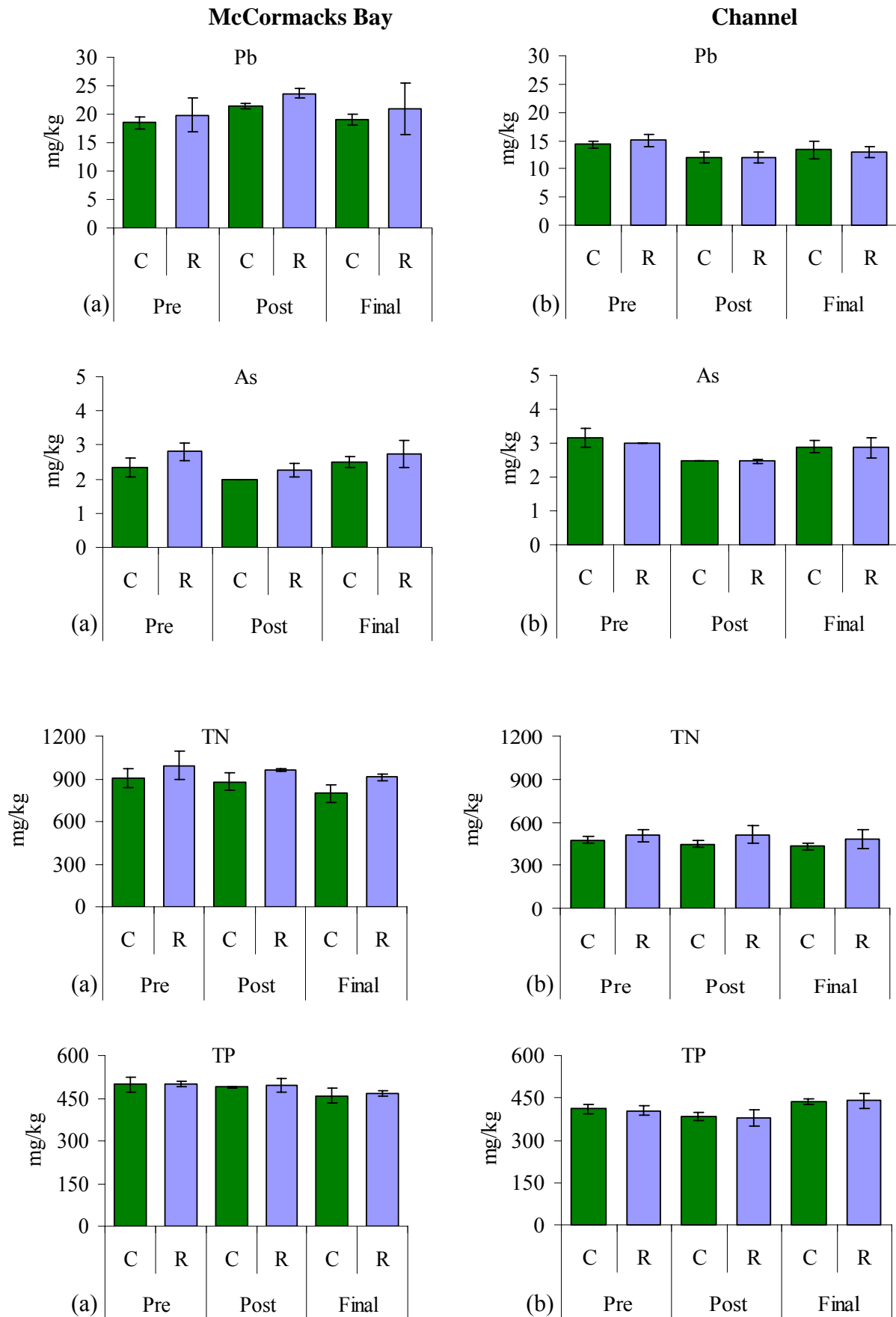


Figure 4.8: Mean (\pm S.D.) concentrations of copper, chromium, zinc, nickel, lead, arsenic, nitrogen and phosphorus from control (C) and removal (R) treatments for each sampling time at McCormacks Bay and the Channel site.

4.3.2 Effects of removing *Ulva lactuca*

Prior to removal at site CH, the cockle bed had a uniform cover of *U. lactuca* mats mixed with numerous empty *A. stutchburyi* shells (Plate 4.1). After removal, bare space increased to ~95% in treated plots (Plate 4.2) and *A. stutchburyi* that were previously covered by *U. lactuca* and empty shells were exposed (Plate 4.2, arrow 1). The majority of empty shells and *U. lactuca* had been swept aside (Plate 4.2, arrow 2) with a few fragments of thallus remaining (Plate 4.2, arrow 3). The day after removal, remaining thallus fragments had been washed away by the tide and numerous *A. stutchburyi* were observed at the sediment-water interface. Removal plots at site MB were effectively clear of *Ulva* spp. and the only obvious epifaunal species present within removal plots was *A. crenata*.

Ulva lactuca biomass

Prior to removal observed overall percent cover of *U. lactuca* was similar at both sites (~50%), although *U. lactuca* dry weight biomass (g) varied within treatments and between treatments as shown by Figure 4.9. There were no significant differences between control plots when comparing the pre- and post-removal samples at site MB or site CH (ANOVA, $P_{(df=1, F=0.03)} = 0.87$) (ANOVA, $P_{(df=1, F=0.33)} = 0.57$) respectively (Fig 4.9a and c). There were significant differences in dry weight biomass (g) of *U. lactuca* between control and removal plots for both sites after removal (Table 4.5). As expected, dry weight biomass (g) decreased significantly in removal plots at this time (Fig. 4.9b and d).

By final sampling at McCormacks Bay, removal plots were recovered by *U. lactuca* (Fig. 4.9b); observed percent cover increased to between 50% and 75% for all plots. There was no significant difference in *U. lactuca* dry weight (g) biomass between treatments at this time (ANOVA, $p_{(df=1, F=1.79)} = 0.18$; Table 4.5). By the final sampling at the Channel site, *U. lactuca* had all but disappeared from both treatments (Fig. 4.9c and d). Within each plot there was an observed cover of < 5%. There was a significant difference in *U. lactuca* dry weight (g) biomass between treatments at this time (ANOVA, $p_{(df=1, F=22.74)} < 0.001$; Table 4.5). Removal plots had less dry weight than control plots (Fig. 4.9c and d).



Plate 4.1: Site CH at low tide looking south before the removal of *Ulva lactuca*.

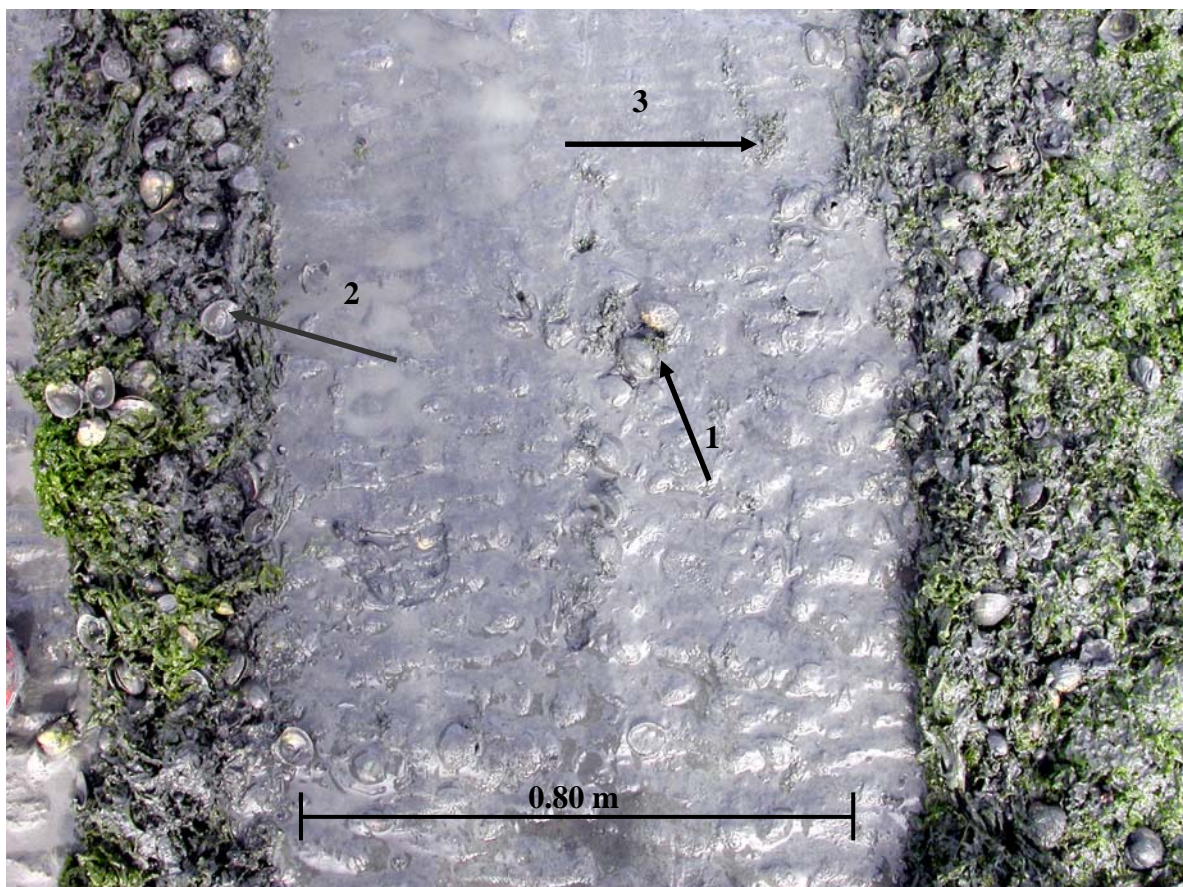


Plate 4.2: Results of removing *Ulva lactuca* with a hand held mechanical broom at site CH. Arrow (1) indicates an exposed live cockle, arrow (2) indicates swept aside empty shells and *Ulva lactuca* and arrow (3) indicates a remaining fragment of *Ulva lactuca*.

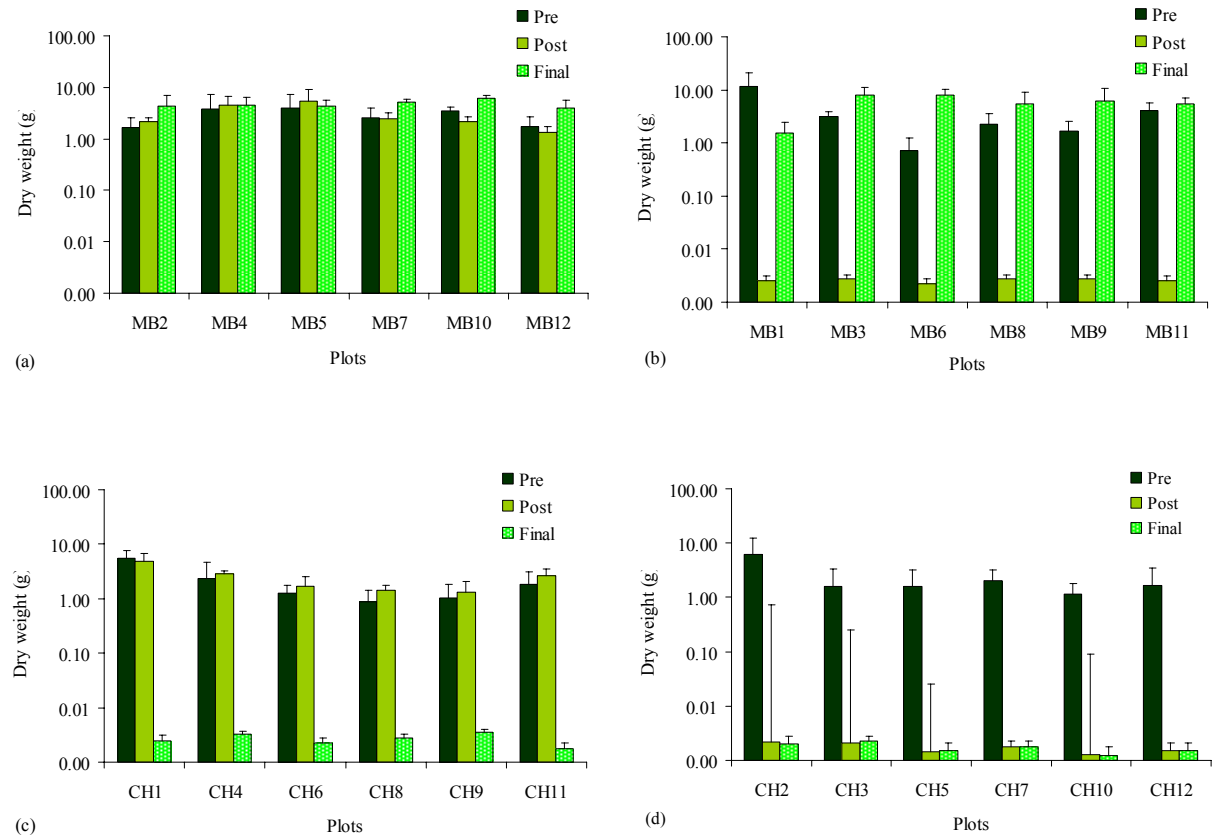


Figure 4.9: Mean (+S.D.) *Ulva lactuca* biomass (g) at (a) site MB control plots, (b) site MB removal plots, and (c) site CH control plots and (d) site CH removal plots for pre- and post-removal, and final sampling time.

Table 4.5: ANOVA results for differences in *Ulva lactuca* biomass between control and removal treatments at each sampling time for McCormacks Bay and the Channel site.

| Time of Sample | McCormacks Bay | Channel |
|----------------|--------------------------------------|--------------------------------------|
| Pre-removal | ANOVA, $p_{(df=1, F=0.78)} = 0.38$ | ANOVA, $p_{(df=1, F=0.09)} = 0.77$ |
| Post-removal | ANOVA, $p_{(df=1, F=45.36)} < 0.001$ | ANOVA, $p_{(df=1, F=53.92)} < 0.001$ |
| Final | ANOVA, $p_{(df=1, F=1.79)} = 0.18$ | ANOVA, $p_{(df=1, F=22.74)} < 0.001$ |

Benthic species abundance

After removal of *U. lactuca* there were significant declines in the mean number of species in removal plots at sites MB and CH (ANOVA, $P_{(df=1, F=6.03)} < 0.05$) (ANOVA, $p_{(df=1, F=16.58)} < 0.001$) respectively (Fig. 4.2). Following this, there was a significant increase in mean number of species between the post-removal and final sampling time at site MB (ANOVA, $p_{(df=1, F=18.78)} < 0.05$; Fig. 4.2). At site CH, there were no significant differences in mean number of species between the pre-removal and final sampling time (ANOVA, $p_{(df=1, F=0.08)} = 0.81$; Fig. 4.2). This indicated recovery in number of species (following the decline after removal) to a similar state as the pre-removal sampling time.

There were significant differences in abundance of species individuals between post-removal treatments at site MB and CH (ANOSIM, $R=0.85$, $p < 0.01$, and ANOSIM, $R=0.49$, $p < 0.01$; Fig. 4.10) respectively. There were no significant differences between treatments at either site for any other sampling time (Table 4.6) as shown by Figure 4.10. At site MB, the significant difference in species abundance between post-removal treatments was shown by SIMPER to be driven by five main taxa. Together they accounted for 55.7% of the variation between treatments. These were the infaunal bivalves *Arthritica bifurca* and adult *Austrovenus stutchburyi*, and the grazing epifauna *Zeacumantus subcarinatus*, *Micrelenchus tenebrosus* and adult *Amphibola crenata* (Table 4.7a). At site CH, 3 taxa were shown by SIMPER to account for 54.9% of the variation between treatments (Table 4.7b). These were the grazing epifaunal *M. tenebrosus*, and the infaunal bivalves *A. stutchburyi* and *Macomona liliana*. At site MB 46 days after removal, the epifaunal species *Z. subcarinatus*, *Z. lutulentus*, *M. tenebrosus* and *H. crassa*, and the infaunal bivalve *A. stutchburyi* (juveniles) had increased in abundance while the remaining species decreased (Table 4.8a). At site CH, infaunal bivalves *A. stutchburyi* (adults) and *M. liliana* and the sea anemone *A. aureoradiata* were the only species that decreased 46 days after removal of *U. lactuca* (Table 4.8b).

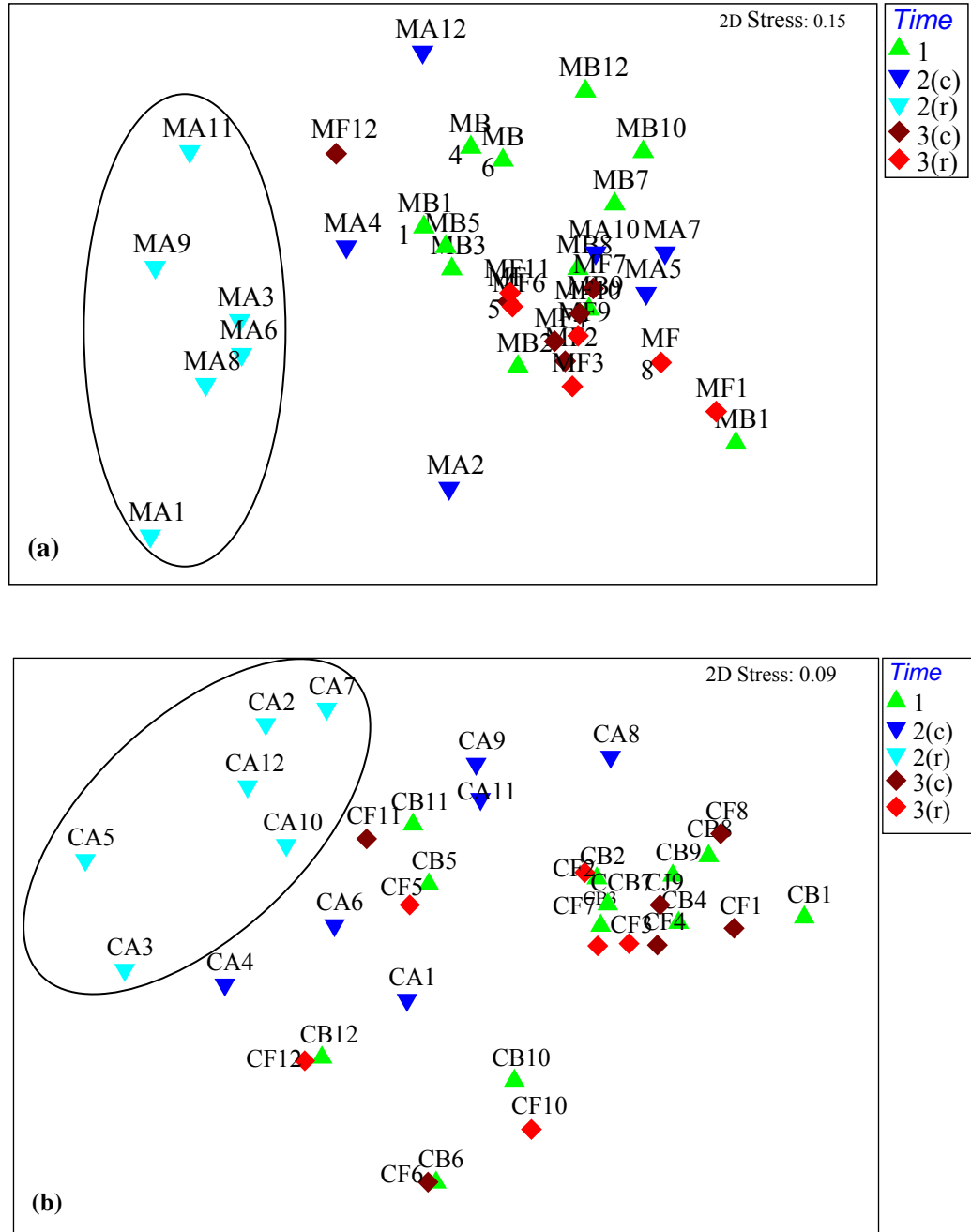


Figure 4.10: MDS ordination of species abundance for the three sampling periods at sites (a) MB and (b) CH. Time 1 (pre-removal), 2 (post-removal) and 3 (final sampling). Removal plots are denoted in the key by r and control plots by c in parentheses. Post removal plots are circled.

Table 4.6: ANOSIM results for differences in species abundance between *Ulva lactuca* control and removal treatments at each sampling time at McCormacks Bay and the Channel site.

| | McCormacks Bay | | Channel | |
|----------------|----------------|---------|---------|---------|
| Time of Sample | R-stat | p-value | R-stat | p-value |
| Pre-removal | 0.067 | 0.22 | -0.089 | 0.78 |
| Post-removal | 0.85 | 0.002 | 0.49 | 0.009 |
| Final | 0.006 | 0.35 | 0.048 | 0.61 |

Table 4.7 : SIMPER results showing mean abundance of each species in control and Removal plots one day after removal at sites (a) MB and (b) CH. Those species that cumulatively contribute to 90% of the variations between treatments are shown. Epifauna with less abundance in removal plots compared to control plots are in bold. Adults = (A), Juveniles = (J).

(a) MB

Average dissimilarity = 39.29

| Species | Habitat | Group Control | Group Removal | Cum. % |
|------------------------------------|----------|---------------|---------------|--------|
| | | Av.Abund. | Av.Abund. | |
| <i>Arthritica bifurca</i> | Infauna | 1.52 | 4.31 | 15.85 |
| <i>Zeacumantus subcarinatus</i> | Epifauna | 6.63 | 3.85 | 29.24 |
| <i>Micrelenchus tenebrosus</i> | Epifauna | 2.03 | 0.00 | 38.71 |
| <i>Amphibola crenata</i> (A) | Epifauna | 2.44 | 2.94 | 47.63 |
| <i>Austrovenus stutchburyi</i> (A) | Infauna | 2.67 | 3.80 | 55.72 |
| <i>Amphibola crenata</i> (J) | Epifauna | 1.30 | 2.14 | 62.48 |
| <i>Cominella glandiformis</i> | Epifauna | 1.87 | 1.76 | 68.61 |
| <i>Diloma subrostrata</i> | Epifauna | 0.98 | 0.77 | 74.05 |
| <i>Anthopleura aureoradiata</i> | Epifauna | 1.21 | 2.28 | 79.34 |
| <i>Zeacumantus lutulentus</i> | Epifauna | 3.87 | 3.28 | 84.61 |
| <i>Notoacmea helmsi</i> | Epifauna | 0.32 | 1.08 | 89.74 |
| <i>Austrovenus stutchburyi</i> (J) | Infauna | 0.40 | 0.63 | 93.35 |

(b) CH

Average dissimilarity = 25.38

| Species | Habitat | Group Control | Group Removal | Cum. % |
|------------------------------------|----------|---------------|---------------|--------|
| | | Av.Abund. | Av.Abund. | |
| <i>Micrelenchus tenebrosus</i> | Epifauna | 6.61 | 3.42 | 25.98 |
| <i>Austrovenus stutchburyi</i> (A) | Infauna | 4.85 | 7.46 | 47.2 |
| <i>Macomona liliana</i> | Infauna | 2.14 | 2.76 | 54.85 |
| <i>Diloma subrostrata</i> | Epifauna | 1.35 | 0.76 | 61.51 |
| <i>Austrovenus stutchburyi</i> (J) | Infauna | 2.26 | 2.57 | 67.55 |
| <i>Helice crassa</i> | Both | 0.77 | 0.84 | 73.05 |
| <i>Arthritica bifurca</i> | Infauna | 0.57 | 0.48 | 78.46 |
| <i>Anthopleura aureoradiata</i> | Epifauna | 3.83 | 3.34 | 83.84 |
| <i>Cominella glandiformis</i> | Epifauna | 0.61 | 0.14 | 88.32 |
| <i>Notoacmea helmsi</i> | Epifauna | 1.53 | 1.18 | 92.61 |

Table 4.8: SIMPER results showing mean change in abundance (positive and negative) in removal plots for each species 46 days after the removal of *Ulva lactuca* at sites (a) MB and (b) CH. Those species that cumulatively contribute to 90% of the variations between treatments are shown. Adults = (A), Juveniles = (J).

(a) MB

Average dissimilarity = 35.20

| Species | Habitat | Post-removal Ave. bund. | Final sample Ave. Abund. | Cum.% |
|------------------------------------|----------|----------------------------|-----------------------------|-------|
| <i>Arthritica bifurca</i> | Infauna | 4.31 | -2.88 | 13.08 |
| <i>Zeacumantus subcarinatus</i> | Epifauna | 3.85 | 6.14 | 24.62 |
| <i>Austrovenus stutchburyi</i> (A) | Infauna | 3.80 | -1.58 | 35.81 |
| <i>Micrelenchus tenebrosus</i> | Epifauna | 0.00 | 1.55 | 43.47 |
| <i>Helice crassa</i> | Both | 0.54 | 2.08 | 51.10 |
| <i>Amphibola crenata</i> (A) | Epifauna | 2.94 | -2.31 | 58.10 |
| <i>Zeacumantus lutulentus</i> | Epifauna | 3.28 | 4.60 | 64.96 |
| <i>Amphibola crenata</i> (J) | Epifauna | 2.14 | -1.4 | 71.41 |
| <i>Cominella glandiformis</i> | Epifauna | 1.76 | -1.64 | 77.55 |
| <i>Notoacmea helmsi</i> | Epifauna | 1.08 | 1.52 | 83.18 |
| <i>Anthopleura aureoradiata</i> | Epifauna | 2.28 | -2.04 | 88.10 |
| <i>Austrovenus stutchburyi</i> (J) | Infauan | 0.63 | 0.99 | 92.9 |

(b) CH

Average dissimilarity = 24.88

| Species | Habitat | Post-removal Ave. Abund. | Final sample Ave. Abund. | Cum.% |
|------------------------------------|----------|-----------------------------|-----------------------------|-------|
| <i>Austrovenus stutchburyi</i> (A) | Infauna | 7.46 | -5.42 | 16.1 |
| <i>Micrelenchus tenebrosus</i> | Epifauna | 3.42 | 5.22 | 31.89 |
| <i>Arthritica bifurca</i> | Infauna | 0.48 | 1.67 | 42.3 |
| <i>Austrovenus stutchburyi</i> (J) | Infauna | 2.57 | 3.63 | 51.23 |
| <i>Notoacmea helmsi</i> | Epifauna | 1.18 | 2.16 | 58.97 |
| <i>Macomona liliana</i> | Infauna | 2.76 | -2.01 | 66.32 |
| <i>Diloma subrostrata</i> | Epifauna | 0.76 | 1.25 | 73.35 |
| <i>Helice crassa</i> | Both | 0.84 | 1.21 | 79.58 |
| <i>Cominella glandiformis</i> | Epifauna | 0.14 | 0.72 | 84.59 |
| <i>Anthopleura aureoradiata</i> | Epifauna | 3.34 | -3.25 | 89.53 |
| <i>Xymene plebeius</i> | Epifauna | 0.13 | 0.48 | 93.12 |

The CI of A. stutchburyi

There were no significant variations in the CI of *A. stutchburyi* between treatments at any sampling time at either site (Fig. 4.11, Table 4.9). By the end of the experiment the mean CI of *A. stutchburyi* had decreased at site MB and increased at site CH (Fig. 4.11). There was a significant difference between the sampling times at site MB (ANOVA, $p_{(df=2, F=4.20)} < 0.05$). This was at the final sampling when there was a decrease in the CI which was greatest in control plots (Fig. 4.11a). At site CH, there were no significant variations between sampling times (ANOVA, $p_{(df=2, F=2.27)} = 0.11$; Fig. 4.11b). No significant variations were found in the

size range of *A. stutchburyi* over time at site MB (ANOVA, $p_{(df=2, F=0.17)}=0.84$; Table 4.10). At site CH, there was a significant difference in the size range between times of sampling (ANOVA, $p_{(df=2, F=6.19)} < 0.01$; Table 4.10); the mean size of *A. stutchburyi* decreased at the post-removal sampling. This was followed by ~100% increase in the minimum range at the final sampling and an increase in the mean size (Table 4.10).

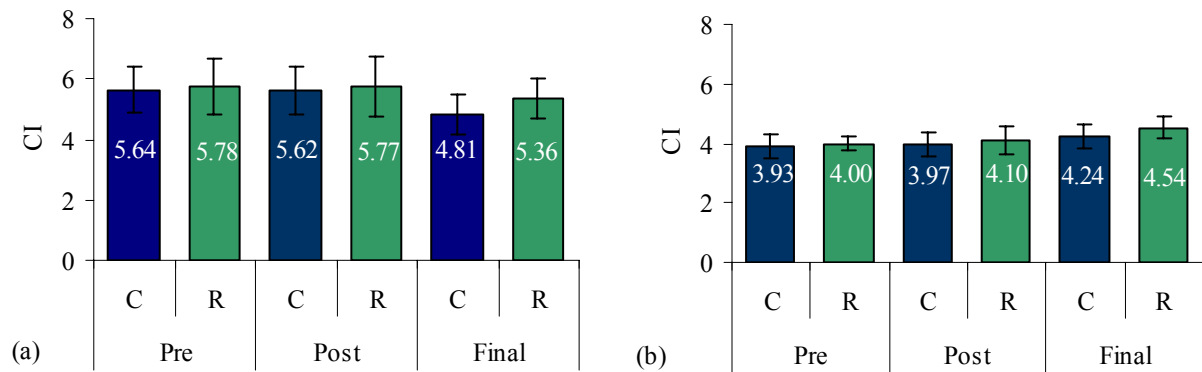


Figure 4.11: Mean (\pm S.E.) CI values for *A. stutchburyi* for each sampling time at sites (a) MB and (b) CH. The mean values are embedded in each bar.

Table 4.9: ANOVA results for variations in the CI of *A. stutchburyi* between *Ulva lactuca* control and removal treatments for each sampling time at sites MB and CH.

| Time of Sample | MB | CH |
|----------------|------------------------------------|------------------------------------|
| Pre-removal | ANOVA, $p_{(df=1, F=0.14)} = 0.71$ | ANOVA, $p_{(df=1, F=0.73)} = 0.40$ |
| Post-removal | ANOVA, $p_{(df=1, F=0.08)} = 0.78$ | ANOVA, $p_{(df=1, F=5.09)} = 0.07$ |
| Final sampling | ANOVA, $p_{(df=1, F=0.05)} = 0.82$ | ANOVA, $p_{(df=1, F=0.02)} = 0.88$ |

Table 4.10: Size and mean range (\pm S.D.) of *Austrovenus stutchburyi* at sites MB and CH for each of the sampling times.

| Time of Sample | MB | | CH | |
|----------------|-----------------|--------------|-----------------|--------------|
| | Size range (mm) | Mean | Size range (mm) | Mean |
| Pre-removal | 10.97-42.94 | 26.52 (6.58) | 15.50-45.40 | 36.50 (7.32) |
| Post-removal | 10.31-42.83 | 25.72 (7.67) | 12.38-43.78 | 32.70 (7.12) |
| Final sampling | 11.48-41.32 | 26.45 (7.92) | 24.08-48.17 | 37.19 (5.62) |

Physico-chemical variables

At site MB, there were significant variations between treatments following removal of *U. lactuca* in temperature and dissolved oxygen of the sediment pore water (ANOVA, $p_{(df=1, F=8.51)} < 0.05$ and ANOVA, $p_{(df=1, F=35.86)} < 0.001$ respectively; Table 4.11a). There were no significant variations between treatments in sediment organic matter, pore water or salinity of pore water following removal of *U. lactuca* (Table 4.12). At site CH, the most significant variation between treatments following removal of *U. lactuca* was in dissolve oxygen of the sediment pore water (ANOVA, $p_{(df=1, F=13555.32)} < 0.001$; Table 4.11b). Two other variables were significant between treatments at this time; temperature ($p=0.05$) and pore water ($p=0.03$) (Table 4.12). At site CH, there were also significant variations between treatments in sediment pore water prior to removal and at the final sampling ($p=0.004$ and $p<0.001$ respectively; Table 4.12). At site MB, prior to the removal of *U. lactuca* there was a significant variation between treatments in dissolved oxygen ($p=0.01$; Table 4.12).

Table 4.11: Mean (\pm S.E.) physico-chemical variables (temperature, dissolved oxygen, salinity, pore water and organics) in control and removal plots at sites (a) MB and (b) CH for the pre- and post-removal, and final time of sampling.

| Site MB | Pre-removal | | Post-removal | | Final | |
|-----------------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Variable | Control | Removal | Control | Removal | Control | Removal |
| Temp ($^{\circ}\text{C}$) | 17.22 (0.02) | 17.23 (0.02) | 16.73 (0.01) | 16.69 (0.01) | 13.28 (0.14) | 13.12 (0.12) |
| D.O. (mg/l^{-1}) | 04.28 (0.05) | 04.51 (0.05) | 04.24 (0.01) | 05.54 (0.01) | 03.13 (0.01) | 03.14 (0.01) |
| Salinity (ppt) | 34.96 (0.37) | 33.36 (1.31) | 32.23 (0.15) | 31.83 (0.19) | 29.78 (0.14) | 29.31 (0.42) |
| Pore water (%) | 30.30 (0.28) | 32.38 (1.34) | 30.41 (0.78) | 31.73 (0.75) | 31.94 (0.85) | 31.92 (0.75) |
| Organics (%) | 03.06 (0.11) | 03.46 (0.18) | 03.20 (0.11) | 03.16 (0.14) | 03.39 (0.22) | 03.27 (0.15) |

(a)

| Site CH | Pre-removal | | Post-removal | | Final | |
|-----------------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Variable | Control | Removal | Control | Removal | Control | Removal |
| Temp ($^{\circ}\text{C}$) | 15.64 (0.02) | 15.70 (0.03) | 17.20 (0.02) | 17.21 (0.02) | 16.39 (0.02) | 16.41 (0.01) |
| D.O. (mg/l^{-1}) | 09.53 (0.02) | 09.54 (0.04) | 09.56 (0.01) | 10.23 (0.01) | 09.55 (0.01) | 09.54 (0.01) |
| Salinity (ppt) | 31.66 (0.23) | 31.63 (0.15) | 31.44 (0.10) | 31.58 (0.05) | 31.15 (0.05) | 31.19 (0.08) |
| Pore water (%) | 24.27 (0.23) | 26.84 (0.67) | 24.27 (0.31) | 25.18 (0.43) | 23.81 (0.39) | 26.60 (0.52) |
| Organics (%) | 02.38 (0.05) | 02.61 (0.06) | 02.11 (0.07) | 02.22 (0.15) | 02.47 (0.06) | 02.25 (0.06) |

(b)

Table 4.12: Summary of ANOVAs ($\alpha=0.05$) comparing the temperature, dissolved oxygen, salinity, pore water, and organics in the control and removal plots at sites MB and CH for each sampling time. * indicates a significant variation between treatments.

| Variable | Time | MB | | CH | |
|-------------------|--------------|-------|---------|-------|---------|
| | | F | p | F | p |
| Temp | Pre-removal | 0.62 | 0.43 | 6.75 | 0.01* |
| | Post-removal | 8.51 | 0.005* | 4.18 | 0.05* |
| | Final | 1.29 | 0.26 | 0.67 | 0.42 |
| D.O. | Pre-removal | 6.48 | 0.01* | 0.14 | 0.71 |
| | Post-removal | 35.86 | <0.001* | 27.32 | <0.001* |
| | Final | 1.57 | 0.22 | 0.98 | 0.33 |
| Salinity | Pre-removal | 0.02 | 0.88 | 0.02 | 0.88 |
| | Post-removal | 0.81 | 0.37 | 2.20 | 0.14 |
| | Final | 0.43 | 0.52 | 0.48 | 0.49 |
| Pore water | Pre-removal | 0.24 | 0.63 | 8.90 | 0.004* |
| | Post-removal | 0.69 | 0.41 | 5.21 | 0.03* |
| | Final | 2.01 | 0.16 | 23.44 | <0.001* |
| Organics | Pre-removal | 0.03 | 0.87 | 3.35 | 0.07 |
| | Post-removal | 0.19 | 0.66 | 0.60 | 0.44 |
| | Final | 2.61 | 0.11 | 0.06 | 0.81 |

At each site there was variation in physico-chemical variables between times of sampling (Fig. 4.12). At site MB, variation was low but shown to be significant between pre- and post-removal (ANOSIM, $R=0.135$, $p<0.001$) compared to the variation between pre-removal and final sampling, and the post-removal and final sampling (Table 4.13). At site CH, the most significant variation was between the pre- and post-removal sampling (ANOSIM, $R=0.854$, $p<0.001$; Fig. 4.12).

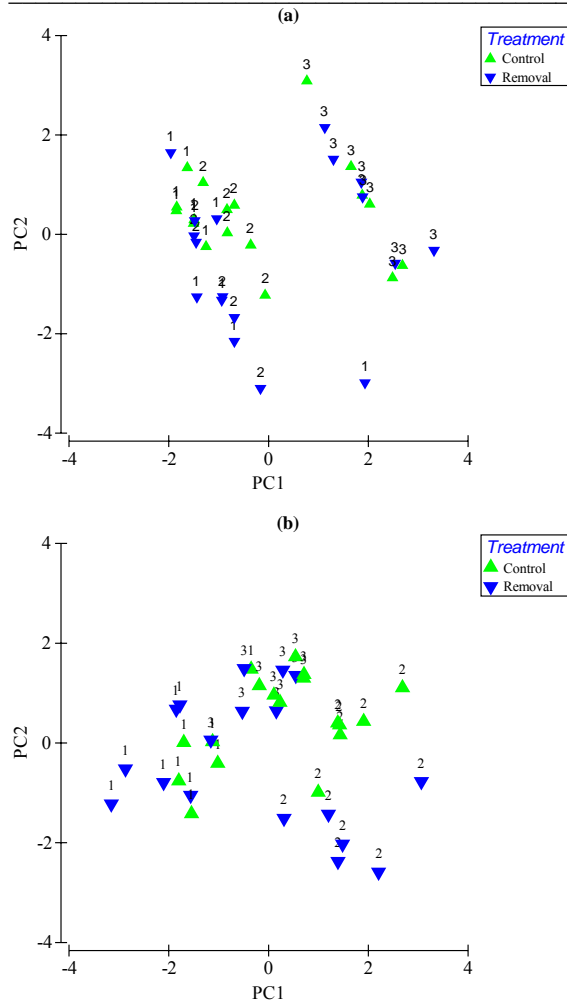


Figure 4.12: PCA of physico-chemical variables for pre (1), post (2) and final (3) times of sampling at sites (a) MB; PC1 and 2 together account for 73% of sample variability, and (b) CH; PC1 and 2 together account for 64% of sample variability.

Table 4.13: Pairwise ANOSIM results for differences in physico-chemical variables between the pre- and post-removal, and final time of sampling at sites MB and CH.

| Time of Sample | MB | | CH | |
|---------------------|-------------------------|---------|-------------------------|---------|
| | Global R=0.571, p<0.001 | | Global R=0.626, p<0.001 | |
| | R-stat | p-value | R-stat | p-value |
| Pre + Post | 0.135 | 0.001 | 0.854 | 0.001 |
| Pre + Final | 0.752 | 0.001 | 0.617 | 0.001 |
| Post + Final | 0.824 | 0.001 | 0.533 | 0.001 |

Sediment composition

There were no significant variations in the percentage of silt/clay between post-removal and final sampling at site MB (ANOVA, $p_{(df=1, F=0.01)} = 0.96$; Table 4.14). At site CH, there was a significant decrease of 40.9% in the mean percentage of silt/clay between pre-removal and final sampling (ANOVA, $p_{(df=1, F=72.66)} < 0.001$) (Fig. 4.13). This ranged from

28.1 to 58.4%. At both sites there were no significant variations in the percentage of silt/clay between treatments at any time (Table 4.15).

Table 4.14: The mean (\pm S.E.) percentage of silt/clay fraction at sites MB and CH site in control and removal plots for pre-, post-removal, and final sampling time.

| | MB | | | CH | | |
|---------|--------------|--------------|--------------|--------------|--------------|--------------|
| | Pre | Post | 6 weeks | Pre | Post | 6 weeks |
| Control | 73.46 (1.46) | 73.85 (1.48) | 76.59 (1.25) | 23.01 (0.46) | 16.95 (0.46) | 13.54 (0.31) |
| Control | 74.61 (1.42) | 74.46 (1.58) | 71.18 (1.34) | 20.01 (0.64) | 09.44 (0.24) | 14.38 (0.37) |
| Control | 64.97 (1.22) | 69.42 (1.32) | 68.72 (1.25) | 22.48 (0.94) | 09.42 (0.12) | 12.69 (0.32) |
| Control | 74.37 (1.80) | 78.74 (1.68) | 72.29 (1.88) | 21.67 (0.40) | 11.35 (0.27) | 12.35 (0.30) |
| Control | 73.12 (1.46) | 76.81 (1.17) | 78.95 (1.73) | 23.04 (0.56) | 09.43 (0.22) | 13.59 (0.34) |
| Control | 72.75 (1.27) | 73.28 (1.38) | 73.01 (1.08) | 21.60 (0.53) | 18.68 (0.55) | 14.85 (0.37) |
| Removal | 75.25 (1.87) | 76.22 (1.70) | 73.95 (1.20) | 19.73 (0.47) | 15.53 (0.27) | 13.40 (0.30) |
| Removal | 74.51 (1.91) | 75.14 (1.52) | 75.73 (1.01) | 23.19 (0.80) | 07.21 (0.25) | 11.30 (0.29) |
| Removal | 63.93 (1.24) | 68.82 (1.26) | 66.04 (1.79) | 17.78 (0.53) | 21.36 (0.75) | 10.93 (0.27) |
| Removal | 82.08 (1.77) | 78.74 (1.68) | 77.29 (1.88) | 31.79 (0.53) | 19.45 (0.60) | 13.23 (0.32) |
| Removal | 79.06 (1.39) | 83.54 (1.47) | 78.14 (1.38) | 23.28 (0.50) | 18.03 (0.46) | 15.68 (0.37) |
| Removal | 74.95 (1.31) | 77.53 (1.33) | 72.39 (1.48) | 27.56 (0.52) | 17.93 (0.43) | 13.37 (0.34) |

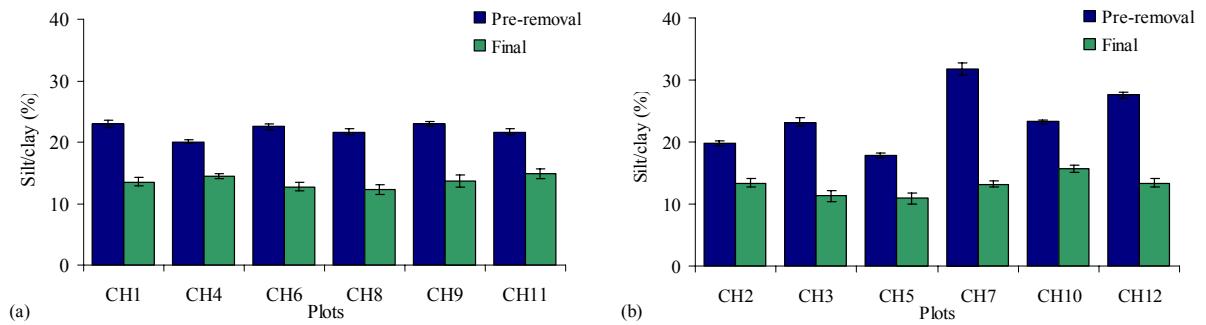


Figure 4.13: Mean (\pm S.E.) percentage of silt/clay fraction at site CH in control (a) and removal plots (b) for pre-removal and final sampling times.

Table 4.15: Summary ANOVA results for variations in the silt/clay fraction between control and removal plots at the pre- and post-removal, and final time of sampling at sites MB and CH.

| Time of Sample | MB | CH |
|----------------|---|---|
| Pre-removal | ANOVA, p ($df=1$, $F=0.89$) = 0.37 | ANOVA, p ($df=1$, $F=0.80$) = 0.39 |
| Post-removal | ANOVA, P ($df=1$, $F=0.90$) = 0.36 | ANOVA, P ($df=1$, $F=2.31$) = 0.16 |
| Final | ANOVA, p ($df=1$, $F=0.04$) = 0.85 | ANOVA, p ($df=1$, $F=0.53$) = 0.49 |

Heavy metal concentrations

At site MB and CH, there were no significant effects of removing *U. lactuca* on concentrations of heavy metals, nitrogen and phosphorus (ANOVA, $p_{(df=1, F=0.02)} = 0.90$ and ANOVA, $p_{(df=1, F=0.02)} = 0.88$ respectively; Table 4.16). There were also no significant differences found between treatments at either site from pre-removal and final sampling (Table 4.16). There were significant variations in the concentrations of heavy metals, nitrogen and phosphorus between times of sampling at site MB and CH (ANOSIM, $R=0.752$, $p=0.001$ and ANOSIM, $R=0.617$, $p=0.001$; Fig 4.14) respectively. At site MB, pre- and post-removal sampling times were grouped closer together than the pre-removal and final sampling and at site CH pre-removal and final sampling were grouped closer together than the pre- and post-removal sampling.

Table 4.16: Summary ANOVA results for variations in the mean heavy metals, nitrogen and phosphorus between *Ulva lactuca* removal and control treatments from each sampling time at sites MB and CH.

| Time of Sample | MB | CH |
|----------------|------------------------------------|-------------------------------------|
| Pre-removal | ANOVA, $p_{(df=1, F=0.02)} = 0.90$ | ANOVA, $p_{(df=1, F=0.003)} = 0.96$ |
| Post-removal | ANOVA, $p_{(df=1, F=0.02)} = 0.90$ | ANOVA, $p_{(df=1, F=0.02)} = 0.88$ |
| Final | ANOVA, $p_{(df=1, F=0.04)} = 0.85$ | ANOVA, $p_{(df=1, F=0.02)} = 0.90$ |

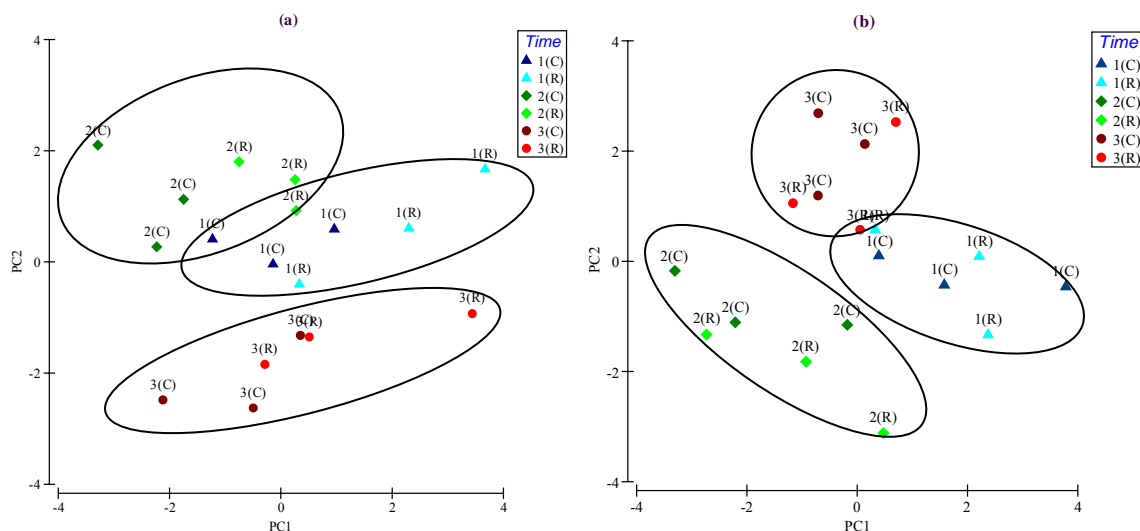


Figure 4.14: Two-dimensional PCA ordination of square-root-transformed and normalised data of heavy metals (chromium, copper, lead, nickel and zinc), and total nitrogen and phosphorus in the sediment for sites (a) MB and (b) CH. 1 = pre-removal, 2 = post-removal, 3 = final sample. Treatments in parentheses are: (C) control (R) removal.

4.4 Discussion

The presence of a single species in high abundance and/or biomass can cause community wide change (Paine 1971; Menge and Sutherland 1976). This observation, that a single species can cause cascading effects on community composition, has been well documented by studies investigating the effects of macroalgal mats in soft sediment estuarine systems (Hull 1987; Everett 1994; Norkko and Bonsdorff 1996b; Raffaelli *et al.* 1998; Lewis *et al.* 2003; Auffrey *et al.* 2004). Because blooms of *U. lactuca* form mats, removal of these was expected to affect species abundance and the sediment physico-chemical environment.

In this study removal of *U. lactuca* mats by hand held mechanical broom resulted in an increase of bare space at both sites to ~100% in treated plots, and biomass (g) of *U. lactuca* reduced to virtually nothing (Fig. 4.9; Plate 4.1 and 4.2). Next day no footprints were visible in plots at the Channel site but were obvious at McCormacks Bay. By the experiments termination mats had recovered removal plots at McCormacks Bay and had totally disappeared at the Channel site. This followed two storm events; the first in early May and the second in mid June. Drifting macroalgal mats at McCormacks Bay had been washed to the site from the eastern side of McCormacks Bay (Fig. 2.1). This area has constant prolific biomass of *U. lactuca*. Mats at the exposed Channel site appeared to have been detached and shredded by shear water stress, stirred up sediments and sharp empty cockle shells all of which are common at this site.

The response of benthic invertebrates to mat removal was species specific. Epifaunal species at both sites were less abundant in removal plots than in control plots and infauna had increased (Table 4.17). The decrease in epifauna was expected as most individuals were swept away by motion of the mechanical broom. Increasing infauna abundance was unexpected because these species are generally sedentary.

Table 4.17: Summary of responses of *Ulva lactuca* biomass, benthic fauna, CI of *A. stutchburyi* and physico-chemical variables 1 day after the removal of *Ulva lactuca* at sites MB and CH. ns= non-significant change. Arrows pointing down represent a significant decrease and arrows pointing up represent a significant increase in biomass, faunal abundance and levels of physico-chemical variables. $\alpha = 0.05$.

| Factor | MB | CH |
|---------------------------|----|----|
| <i>U. lactuca</i> biomass | ↓ | ↓ |
| Epifauna mean abundance | ↓ | ↓ |
| Infauna mean abundance | ↑ | ↑ |

| | | |
|-----------------------------|----|----|
| CI of <i>A. stutchburyi</i> | ns | ns |
| Dissolved oxygen | ↑ | ↑ |
| Salinity | ns | ns |
| Organic matter | ns | ns |
| Pore Water | ns | ↑ |
| Silt/ clay | ns | ns |
| Temperature | ↑ | ↑ |
| Copper | ns | ns |
| Lead | ns | ns |
| Arsenic | ns | ns |
| Nitrogen | ns | ns |
| Phosphorus | ns | ns |
| Chromium | ns | ns |
| Nickel | ns | ns |
| Zinc | ns | ns |
| Cadmium | ns | ns |

At McCormacks Bay, there were significant effects on benthic invertebrates in removal plots (Table 4.7a) with twelve species contributing to 90% dissimilarity between control and removal treatments. Of these, only two were infaunal species: *A. bifurca* and *A. stutchburyi* (Table 4.8). Low abundance of these two species (Table 4.3) could have been as a direct consequence of low dissolved oxygen, high silt/clay fraction (Table 4.4), and biomass of *U. lactuca* mats at the site (Fig. 4.4a). This is consistent with the effects of macroalgal mats proposed by Hull (1987) (Fig. 4.1), as reported by Everett (1994) and as discussed by Raffaelli *et al.* (1998). Higher abundance of the more mobile epifaunal species *A. crenata* in removal plots compared with control plots (Table 4.7a) is consistent with findings of a removal study in southern Ireland where epifaunal species quickly immigrated to removal plots (Lewis *et al.* 2003). Decrease in *Z. subcarinatus* and *Z. lutulentus* in removal plots was expected because they were usually found feeding on *U. lactuca* and were less likely to immigrate from control plots to bare space. However, finding individuals of *Z. subcarinatus* and *Z. lutulentus* in the post-removal samples was surprising as these epifaunal

species were not visible during the post-removal sampling process. It was thought that individuals had been forced into the soft sediments of McCormacks Bay by the removal process.

It is thought that benthic communities, especially infauna, of energetic sandy habitats have faster recovery rates from physical disturbance than those from depositional muddy sediments (Schratzberger and Warwick 1999; Ferns *et al.* 2000; Dernie *et al.* 2003). At the Channel site, there was less effect of removal on benthic invertebrates compared to McCormacks Bay (Table 4.7). This could be attributed to the dominance of sand which is more compacted compared to the softer sediments that dominate McCormacks Bay (Table 4.4). The sandier sediments were relatively undisturbed at the Channel site by the removal process that included trampling compared to the softer heavily disturbed sediments at McCormacks Bay. Trampling was the most visible cause of disturbance at McCormacks Bay where footprint impressions were ~ 15 cm deep. This may have compounded the effect of removal on invertebrates at this site. At the Channel site footprint impressions were less than 1 cm deep (Plate 4.2) and were less likely to affect benthic species. Two months after termination of the removal experiment at McCormacks Bay substantial biomass of the filamentous alga *Rhizoclonium* spp. were observed in areas of high disturbance. These areas included access points across the mudflats and around plots. This indicated that *Rhizoclonium* spp. may be an early coloniser of disturbed areas and may act as a facilitator for colonisation of other benthic species. Some months later *U. lactuca* dominated these areas. It was thought that the filamentous *Rhizoclonium* spp. had been out-competed and smothered by the laminar *U. lactuca*.

There was no significant effect of removing *U. lactuca* mats at either site on condition (CI) or size of *A. stutchburyi*. This was shown by the similarity of the CI between times of sampling (Fig. 4.11) and between treatments at both sites (Table 4.9). These findings were consistent with those of Maclaren (2005). Following removal of *U. lactuca*, *A. stutchburyi* were found to be more abundant in post-removal plots at both sites compared to control sites (Table 4.7). This coincided with increased dissolved oxygen in removal plots at each site. Interestingly Hull (1987) found greater numbers of the bivalve *Macoma balthica* under experimentally increased macroalgal mats compared to control areas. A possible explanation for this is that he used *Enteromorpha* spp., a filamentous form of macroalgae that is unlikely to have smothering effects on the sediments and biota as the laminar *U. lactuca*. It is unlikely that increased levels of dissolved oxygen at each site were the reason for higher abundance of *A. stutchburyi* as they are mostly sedentary; individuals have been reported to move from a few centimetres to a metre (Mouritsen 2004). However, they would have to travel over

several meters from nearby untreated areas within 24 hours of removal. The more likely explanation is that burial depth of these bivalves was reduced by the sweeping action of the mechanical broom. This removed upper layers of soft sediments at McCormacks Bay and layers of empty shells at the Channel site resulting in greater numbers of live individuals in each sample.

Forty six days after removal, adult *A. stutchburyi* (>21mm) had decreased in abundance at both sites and juveniles (<20mm) had increased (Table 4.8). Based on effects of macroalgal mats on invertebrates by Hull (1987; Fig. 4.1) and personal observations, the removal of the physical barrier (macroalgal mats) may have allowed access to mudflat predators such as shore birds, and whelks. These may have been responsible for declines in *A. stutchburyi* abundance at both sites (Hull 1987). At the Channel site, shore birds such as the pied oystercatcher (*Haematopus finschi*) and the eastern bar-tailed godwit (*Limosa lapponica*) were regularly seen foraging in greater abundance on removal plots than control plots. These birds are thought to predate on larger individuals following the optimal foraging theory as empty shells at the Channel site were generally large (pers. obs.). Fish such as the sand flounder (*Rhombosolea plebeia*) may also have been restricted by *U. lactuca* mats from feeding on *A. stutchburyi* (Isaksson *et al.* 1994). Interestingly, once *U. lactuca* had been removed, red-billed gulls (*Larus novaehollandiae*) were observed foot-paddling (a foraging behaviour that disturbs small invertebrates that are then fed upon) only within cleared plots at the Channel site. This indicated that macroalgal mats also prevented predation on smaller mobile invertebrates which is consistent with the observations of Lewis *et al.* (2003). Rapid recovery of benthic assemblages at each site following the removal experiment was expected; Hall and Harding (1997) found that recovery took 56 days during a study on the effects of tractor and hydraulic suction dredging in Solway Firth, United Kingdom. This process is much more invasive than merely sweeping the surface of sediments with a mechanical broom.

Dissolved oxygen of sediment pore water increased significantly at both sites the day after removal (Table 4.17) and is consistent with the study by Bolam *et al.* (2000). This is also the opposite effect following development of macroalgal mats where dissolved oxygen decreases (Fig. 4.1; Hull 1987). This was shown at McCormacks Bay where dissolved oxygen decreased by the final sampling when macroalgal mats had recovered all removal plots (Table 4.11). There is a probability that increase at both sites following the removal treatment resulted from disturbance to sediments by the mechanical broom rather than direct exposure to oxygenated water during the following high tide. Supporting evidence for this is the similarity between lower levels of dissolved oxygen from pre-removal and final sampling at the Channel site compared to the higher levels recorded at the post-removal sampling (Table

4.11). The higher levels of dissolved oxygen at the Channel site compared to McCormacks Bay may have been attributed to the higher wind and wave action which can cause increased oxygen in sediments even with algal cover (Baillie 1986). However, in summer especially in shallow water, dissolved oxygen in sediments is low. This is because of the low solubility of oxygen in water at higher temperatures compared to colder temperatures (Rasmussen and Jorgensen 1992). This may also have contributed to the differences between sites as the temperatures in McCormacks Bay are generally higher than at the Channel site.

At both sites, significant variations in pore water temperature between treatments would be explained by direct exposure of the sediments to heating from the sun after removal of *U. lactuca*. At the Channel site, the significant variation in sediment pore water between treatments was common to all sampling times (Table 4.12). Therefore, it is unlikely to be as a direct result of the removal treatment.

Although sediment composition was not significantly affected by the initial removal of *U. lactuca* at either site, there was a significant decrease of ~40% in the silt/clay fraction in all plots at the Channel site by the final sample in June (Fig. 4.13). This may have resulted from loss and decay of *U. lactuca* leading to increased water velocity, decreased silt deposition and increased flushing at this site (Escartin and Aubrey 1995). Also, two storm events that occurred in early May and mid June would have caused strong vertical mixing in the shallow water column. This may have contributed to the decreased silt/clay fraction at the exposed Channel site.

Differences in concentrations of heavy metals between times of sampling at each site (Fig. 4.14) may be attributed to natural variations from sources of heavy metals such as: organic matter, natural rock detritus and precipitation of sulphides, carbonates, hydroxides and phosphates of heavy metals from alkaline seawater (Dyer 1979; Forstner 1979; Turner *et al.* 1998). Estuarine sediments also act as a sinks for nitrogen and phosphorus, and are traps for heavy metals including chromium, copper, nickel and lead which are found in the silt/clay fraction where sorption and cation exchange occurs during oxidation (Dyer 1979; Nowicki and Nixon 1985; Rizzo and Christian 1996; Trimmer *et al.* 1998). Release of nitrogen and phosphorus back to the water column has been well established (Nowicki and Nixon 1985; Cowan and Boynton 1996; Rizzo and Christian 1996; Clavero *et al.* 2000). This may have contributed to significant differences in concentrations of these nutrients between times of sampling (Fig. 4.8 & Fig. 4.14). Resuspension or reworking of sediment heavy metals by physical processes such as strong vertical mixing and tidal action may also have contributed to differences in concentrations over time (Cundy *et al.* 2003; Rosales-Hoz *et al.* 2003). Other sources that may have contributed to these variations include runoff from urban, industrial

and agricultural practices within the watershed (GESAMP 2001). These practices include use of arsenical pesticides, fertilisers and combustion of fossil fuels (Valette-Silver *et al.* 1999). Atmospheric deposition of combusted fossil fuels such as lead to marine sediments is another possible source of variation (Munoz and Salamanca 2003). Deposition of nitrogen and other nutrients from atmospheric and groundwater sources may also be considered as factors contributing to variations over time (Paerl 1997). Finally leaching of heavy metals from landfills in the vicinity can cause fluxes in contamination levels (Knox and Kilner 1973).

Prolific abundance of *U. lactuca* in McCormacks Bay may be attributed to high levels of nitrogen in sediments and this species ability to intercept fluxes of nutrients from the sediment (Tyler *et al.* 2001). Decay of algae within the system would contribute to nutrient levels thus locking the enclosed system into an undesirable enriched nutrient state where *U. lactuca* would not be limited (Fong 2000; Troell *et al.* 2005). The sheltered and shallow nature of the Bay may also contribute to the persistence of *U. lactuca*. This would provide protection from desiccation by wind and tidal action. The calm conditions in the Bay would lead to better water clarity and light availability resulting in optimum growth conditions (Coutinho and Zingmark 1993; Vergara *et al.* 1997).

Summary and conclusions

Removal of *U. lactuca* mats resulted in decreased abundance of epifaunal and an increase of infaunal species and no effect on the CI of *A. stutchburyi*. Over a relatively short period species abundance returned to a similar state of that prior to removal. Physico-chemical variables were not significantly affected by removal of *U. lactuca* with exception of dissolved oxygen and temperature. The greatest visible impact during the experiment was from disturbance to sediments in McCormacks Bay by the removal technique and trampling. The site continued to show evidence of disturbance some months after termination of the experiment with pooling of water in depressions formed by trampling.

It was concluded that removal of *U. lactuca* by a hand-held mechanical broom was an effective method that had little impact on benthic invertebrates and physico-chemical variables in areas with a low silt/clay fraction. However, it is recommended that a large scale experiment be conducted as results of removal may be scale dependent; large scale removal may have a greater impact with longer lasting severe effects. In McCormacks Bay, or other

areas high in silt/clay, it is recommended to use an alternative removal technique. This would ideally be undertaken from a boat during high tide to avoid trampling the mudflats and reduce the impact to benthic invertebrates. Removal at high tide would assist in the detached shreds of *U. lactuca* being washed out to sea rather than being retained within the Estuary. The use of a hydraulic suction device in McCormacks Bay may be appropriate as recovery by benthic fauna from this type of disturbance has been shown to be within a relatively short period (Hall and Harding 1997).

The development of a modelling program such as the estuary-wide MOHID system, which has been applied mainly in Portuguese estuaries, would focus management efforts of *U. lactuca* in the future (Trancoso *et al.* 2005). The model should include the physico-chemical profiles of seed sites, past trends in *U. lactuca* biomass, and spore production and dispersal within the Avon-Heathcote Estuary.

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(Tallqvist 2001)

Chapter 5

General discussion

Globally, estuarine ecosystems are under increasing pressure from anthropogenic eutrophication, particularly from direct discharge of treated sewage high in the macronutrients nitrogen and phosphorous (Nixon 1995; Richardson and Jorgensen 1996; GESAMP 2001). This results in primary and secondary symptoms of eutrophication such as problematic macroalgal growth which leads to nuisance blooms (Bricker *et al.* 2003). These blooms have negative effects on benthic invertebrates by creating hostile environments in and under the mats (Hull 1987; Bonsdorff 1992; Everett 1994; Raffaelli *et al.* 1998; Bolam and Fernandes 2002). When detached, washed ashore and decomposing, the unpleasant smelling gas hydrogen sulphide is produced (Soulsby *et al.* 1982; Valiela *et al.* 1997; Raffaelli *et al.* 1998). This becomes a nuisance to local residents and recreational users of estuarine systems. Local authorities are then under pressure from the public to manage and control these nuisance blooms by implementing long and short term remediation strategies (Bettinetti *et al.* 1996; Valiela *et al.* 1997). Long term strategies include reduction of pollution and diversion of sewage outfalls, short term strategies include removal of shoreline accumulations of washed up macroalgae or mats from intertidal areas (Bettinetti *et al.* 1996; Valiela *et al.* 1997).

This thesis investigated benthic community assemblages at seven sites in the Avon-Heathcote Estuary across sediment physico-chemical gradients to determine patterns of spatial and temporal distribution. Dispersal, settlement and growth of *Ulva lactuca* L. zoospores were investigated in areas of the Estuary by placement of artificial substrates. The effects of mechanically removing *U. lactuca* from intertidal sand and mudflats on benthic invertebrates and sediment physico-chemistry were also investigated. These studies were conducted because *U. lactuca* are important in structuring benthic communities of the Avon-Heathcote Estuary and have become a nuisance to local residents and recreational users over the past few decades (Knox and Kilner 1973; Steffensen 1974; Bressington 2003).

The remainder of this concluding chapter is organised as follows: Firstly, major findings of chapters 2, 3 and 4 are summarised. Individual chapters include detailed and specific discussions of results and only major points will be repeated in this chapter. Secondly, I outline findings of studies that recommended strategies for management and control of macroalgal blooms and some positive and negative effects of removing macroalgal

mats. Thirdly, I comment on importance of scale in relation to disturbance and experimental treatments. Fourthly, I discuss the Christchurch City Council's plans for the Avon-Heathcote Estuary in relation to sewage outfall diversion and alteration to the culvert at McCormacks Bay, and possible effects on distribution and abundance of *U. lactuca*. I conclude with a summary of future directions and recommendations for research needs that arise from this thesis.

Major findings

The Saturn DigiSizer was found to be a consistently accurate method for analysing sediment particle size that saved time and effort. This method was also found to be comparable to the traditional sieve/pipette method. As such, this would allow for a confident comparison between results of the current study using DigiSizer and previous and future studies using the traditional pipette method.

The mechanical broom was found to be a highly portable and efficient tool for removing *U. lactuca* mats with low impacts to the benthic invertebrates and sediments on intertidal sandflats but not intertidal mudflats. The mudflats were too difficult to traverse; the operator sank regularly and struggled to keep balance which made using the mechanical broom difficult.

Examination of the benthic invertebrates found in the present study revealed they belonged to the following groups: Decapoda, Gastropoda, Bivalvia, Polychaeta and others that include Polyplacophora, Anthozoa, Sipuncula, Amphipoda and Cirripedia. The three most abundant species were the infaunal bivalve *Austrovenus stutchburyi*, and the epifaunal gastropods *Micrelenchus tenebrosus* and *Amphibola crenata*. These species were found to have a wide distribution that varied over spatial and temporal scales. This study demonstrated that variations in community assemblages occurred seasonally at local scales. These variations were correlated with sediment physico-chemical variables at each site including: temperature, dissolved oxygen, pore water, salinity, organic matter and the sediment particle size but not *U. lactuca* biomass. These results are summarised in Table 5.1. The conclusion from the comparative study was that natural variability occurred in both the community assemblages and sediment physico-chemical variables between the years of the studies. However, the amount of variability could not be accurately determined because of the differences in sample sizes used in each study.

Table 5.1: Results of the BIOENV analysis for individual sites and the physico-chemical variables that correlated best with the patterns in community structure (refer to Fig. 3.6).

| Site | Environmental variables | Rho-value |
|-----------------|---------------------------------------|-----------|
| Causeway | D.O and organics | 0.657 |
| Channel | Salinity | 0.771 |
| Raupo Bay | Temp and pore water | 0.771 |
| Ebb Tide Street | Temp and organics | 0.657 |
| Penguin Street | Temp, pore water, silt/clay | 0.714 |
| Tern Street | Temp, pore water, organics, silt/clay | 0.943 |
| McCormacks Bay | Temp | 0.314 |

By analysing changes in benthic invertebrate assemblages, their abundance and sediment physico-chemistry over temporal scales, the effects of removing *U. lactuca* mats from intertidal sand and mudflats were determined. The significant responses immediately following the removal treatment are summarised in Table 5.2. The results indicated that abundances of epifauna were negatively affected by the removal treatment and this was attributed to individuals being swept from the plots. Infaunal abundances increased at each site. This was attributed to the removal of surficial sediments and layers of empty shells exposing more individuals to collection.

The recovery of assemblages, (approached similarity to the pre-treatment state) six weeks following removal, indicated that at a small scale, removal of *U. lactuca* mats had a short term effect on benthic invertebrates. The increase in dissolved oxygen was attributed to the disturbance of sediments from the rotating drum and fins of the mechanical broom. Temperature increases of the sediment pore water were thought to be a consequence of exposure to heating from the sun.

Table 5.2: Summary of significant responses one day following the removal of *Ulva lactuca* mats at McCormacks Bay and Channel site. Arrows pointing down represent a significant decrease and arrows pointing up represent a significant increase in biomass, faunal abundance and levels of physico-chemical variables. $\alpha = 0.05$.

| Factor | McCormacks Bay | Channel |
|-----------------------------|----------------|---------|
| <i>Ulva lactuca</i> biomass | ↓ | ↓ |
| Epifauna mean abundance | ↓ | ↓ |
| Infauna mean abundance | ↑ | ↑ |
| Dissolved oxygen | ↑ | ↑ |
| Temperature | ↑ | ↑ |

In the present study, settlement and growth of *U. lactuca* zoospores was dependent on availability of cockleshells as suitable substrates. Settlement was thought to be related to hydrophobicity (greater numbers of spores attach with increasing angle of an object (Callow *et al.* 2000) because no zoospores were found to have settled on flat control tiles. Zoospores were thought to have a limited dispersal from seed sites because of hydrodynamics and currents within the Estuary. Burial of viable thallus in sediments over winter, and their later release in spring, was thought to be a mechanistic explanation for the appearance of attached plants at sites far from major seed sources.

Critique of methods

Difficulties relating to determination of sample sizes and collection methods are prevalent in ecological studies. While this study was relatively small in scale, preliminary work to determine accuracy of methodology was undertaken, and this indicated that the techniques and sample sizes used were adequate to answer the questions posed. However, it should be noted that a large scale removal study might offer very different results regarding effects on benthic invertebrates and their recovery rates.

Other physical variables could have been included but were not for reasons of time, resources and because they were less closely related to the central questions of the thesis. Examples include correlation of *U. lactuca* biomass with column water salinity and temperature, current velocity, column water turbidity and chlorophyll a content of the sediments at each site. Increasing the sample size to six replicates at each site during general surveys would contribute to an increased understanding and definition of patterns between community assemblages and physico-chemical variables at the local scale. This was indicated by the results of the quadrat assessment (Chapter 2, Section 2.8) and observations in the field concerning clumping of estuarine invertebrates.

Management and control of macroalgal blooms

Blooms of nuisance macroalgae occur frequently in coastal ecosystems affected by eutrophication (Soulsby *et al.* 1982; Hull 1987; Everett 1991; Runca *et al.* 1996; Hernandez *et al.* 1997; Franz and Friedman 2002). In response to this problem, evaluation of management strategies, such as reducing nitrogen loadings in four US east coast estuaries, have been conducted (Whitall *et al.* 2004). Also, reviews of eutrophication in the Lagoon of Venice, Italy (Bettinetti *et al.* 1996) and Waquiot Bay, Massachusetts (Valiela *et al.* 1997) led to proposed management and regulation strategies for macroalgal blooms. Management

recommendations common to these studies included a three phase strategy; the reduction of pollution, (long term), modification of water circulation, (medium term), and removal of macroalgal biomass, (short term).

Long term management strategies such as the reduction of pollution by developing waste-water management schemes and diversion of intertidal sewage outfalls have reduced the level of eutrophication and the associated effects such as nitrification in the Bilbao Estuary, Spain (Oreja and Salinas 2003) and the Fraser River Estuary, Canada (Arvai *et al.* 2002). The city of Bilbao on the north coast of Spain has a population of approximately one million. The Bilbao Estuary is 15 km long, has freshwater inflows of 25 m³/s and has been used for disposal of large volumes of domestic and industrial effluents. The strategy to reduce eutrophication involved developing a sewage plan that incorporated networks of interceptor and collection sewers with major waste-water treatment plants and was completed in 2002 (Oreja and Salinas 2003). The local water authority envisaged biological recovery of native fauna after a substantial increase in dissolved oxygen to normal levels of approximately 6.3 mg/l⁻¹.

The use of the Watershed Assessment Tool for Evaluating Reduction Strategies for Nitrogen (WATERSN) model in four US east coast estuaries showed that biological removal of nitrogen in waste-water treatments produced the greatest reduction of nutrient loading by 32-57%. This is applicable to watersheds similar to the Avon-Heathcote Estuary's where watersheds are primarily urban (Table 1.3) (Whitall *et al.* 2004). However, estuarine disposal of treated effluents still contributes to eutrophication. Primary-treated sewage effluent from Vancouver, Canada was discharged directly onto intertidal mudflats of the Fraser River Estuary between 1962 and 1988. This resulted in formation of a large azoic zone on the mud and sand flats of Sturgeon Bank. In 1988, as a response to public and agency concern regarding effects on the ecosystem, the Greater Vancouver Sewerage Drainage District (GVSD) constructed a subtidal outfall. Effluent is presently discharged at a depth of 100 m in the Strait of Georgia, five km seaward of the original outfall (Arvai *et al.* 2002). Studies were conducted between 1994 and 1996 to determine the extent of ecosystem improvement following sewage diversion and compared to studies prior to the diversion. Results showed considerable improvement to the mudflat ecosystem including increased dissolved oxygen, decreased sediment chlorophyll, decreased organic material in sediments, reduced heavy metals in surficial sediment, increased grain size, and re-colonisation of the previously azoic zone by the amphipod *Corophium salmonis*. This was important because *C. salmonis* is a primary food source for young Chinook salmon (*Oncorhynchus tshawytscha*) and the Fraser

River Estuary is a nursery habitat for the largest wild population of *O. tshawytscha* in the world (Arvai *et al.* 2002).

Positive and negative effects of removing macroalgal mats

Removal of macroalgal mats is a short-term management strategy that does not reduce eutrophication but resolves initial negative effects of the mats. These effects include: reduced species diversity and abundance, increased sedimentation, sediment anoxia, reduced light penetration and disappearance of eelgrass (Bettinetti *et al.* 1996; Valiela *et al.* 1997; Whitall *et al.* 2004).

There are positive and negative impacts of removing macroalgae from estuarine ecosystems (Wennhage and Pihl 1994; Bonsdorff *et al.* 1997). In Po River Delta, Italy, the reason for removing macroalgae blooms was to protect valuable commercial shell fisheries from *Ulva* spp. deoxygenating the water (Cellina *et al.* 2003). In the Peel-Harvey Estuary in Western Australia, harvesting was used as a tool for managing nuisance macroalgal blooms and resulted in initial negative effects. These included a reduction in macrophyte detritus and declines of fish and benthic epifauna. However, within two months, these variables recovered and resembled non harvested areas (Lavery *et al.* 1999). Juvenile plaice (*Pleuronectes platessa* L.) have been shown to prefer algal-bare sediment compared to algal-covered sediment. Therefore, removal of macroalgal mats would have a positive effect for this species (Wennhage and Pihl 1994). Mats may also act as a refuge for small inshore fish and removing the macroalgae could result in loss of some species (Bonsdorff *et al.* 1997). Drifting mats were reported to facilitate transport of invertebrates and provide alternative habitat for fauna. Removal of these mats would result in a negative effect on these fauna (Norkko *et al.* 2000; Salovius *et al.* 2005).

The Zandvlei Estuary, South Africa, has recreational and residential value that was threatened by the aquatic plant *Potamogeton pectinatus* L. which formed dense beds in response to high nutrient levels. The plant also provided a substratum for the attachment of nuisance algae such as *Enteromorpha intestinalis* (L.) and *Cladophora* spp. In other systems, the plant is important because it increases water transparency, water column oxygenation and substratum for benthic fauna. The associated epifauna were reported to be important in maintaining water quality and ecosystem functioning (Stewart and Davies 1986). In response to proposed control and management of the plant, two harvesting techniques were trialled; controlled height harvesting to 0.8 m below water surface and regular harvesting to the maximum depth of the harvester at 1.7 m depth. It was concluded that both techniques were detrimental to benthic fauna standing stock and that any management program should include

conservation of undisturbed areas to preserve important benthic fauna and water quality (Stewart and Davies 1986). This should be considered in respect to control of *U. lactuca* in the Avon-Heathcote Estuary as *Ulva* spp. is a natural component of estuarine ecosystems and as such promotes heterogeneity of the habitat (Raffaelli *et al.* 1998).

In the present study, mechanical removal of macroalgal mats resulted in an immediate significant decline in abundance of benthic invertebrates. Forty six days following removal, benthic invertebrates at each site had recovered to a similar state as the pre-removal plots. This is consistent with the findings of the study in Peel-Harvey Estuary by Lavery *et al.* (1999), who reported minimal effects of mechanically removing *Ulva* spp. mats on benthic invertebrates.

Disturbance to the sediments was greater in the soft silt/clay sediments of McCormacks Bay than in the more central Estuary Channel site. Recovery at McCormacks Bay was observed two months following the mechanical removal experiment. This was consistent with the study by Hall and Harding (1997), who examined effects of two methods of mechanically harvesting cockles on non-target benthic infauna: hydraulic suction and tractor dredging. This was conducted at Auchencairn Bay, Solway Firth, Scotland in 1993. They found that fauna recovered from the suction dredging within 56 days and that the most likely mechanism of recovery was immigration of adults into disturbed areas. The conclusion was that both mechanical harvesting methods resulted in high mortality levels on non-target benthic fauna and that recovery of disturbed sites was rapid with low overall effects on assemblages.

Scale of disturbance and treatment

The observation that a single species in high abundance and/or biomass is an important mechanism in structuring community composition has been well documented by studies investigating the effects of macroalgal mats in estuarine systems throughout the world (Hull 1987; Everett 1994; Norkko and Bonsdorff 1996; Raffaelli *et al.* 1998; Lewis *et al.* 2003; Auffrey *et al.* 2004). Disturbance is also an important mechanism in the structuring of communities (Paine 1971). Disturbance is an event that disrupts community structure, available resources, substrate availability or the physical environment (Krebs 2001). Frequency of natural disturbance can be periodic (“pulse”) or persistent (“press”). Communities can recover from periodic disturbance. However, if disturbance is persistent, then recovery to a pre-disturbed state is unlikely (Krebs 2001). Therefore, the period and extent of the effect on communities of a single removal event of a species such as *Ulva* spp. (“pulse disturbance”) can be determined by the reduction or loss of non-target species and

their recovery. Conversely, if removal events are regular (“press disturbance”), effects on non-target species may be persistent and recovery may be indeterminate (Bender *et al.* 1984; Keough and Quinn 1998).

In the present study removal of *U. lactuca* was a “pulse” disturbance. Therefore, the resulting effects of removal on the benthic invertebrates and sediment physico-chemical variables are only applicable to a single control event of *U. lactuca*. Impacts of a regular control of nuisance blooms have not been examined in this study. Therefore, an important opportunity exists for future research to address the effects of “press” disturbance on the benthic communities and intertidal sand and mud flats of the Avon-Heathcote Estuary.

Scale is also important, and difficulties arise when making generalisations from the effects of manipulative experiments such as in the removal of *U. lactuca* in the present study. This is because a principal difficulty of field studies is the interpretation of results from an experiment at a particular scale in relation to the general environmental effects (Thrush *et al.* 1997; Thrush *et al.* 2000). This results in a need to compromise between maximising the spatial scale (plot sizes) to best match real world scenarios (removing extensive macroalgal mats) and retaining the amount of replication needed for statistical rigour and detecting fine scale effects (Thrush *et al.* 2000). In field studies, this is often determined by time, financial and resource constraints (Schneider 1994; Quinn and Keough 2002).

In this study, the scale of the removal experiment at each site was local but not broad. This allowed for sufficient replication of treatments while retaining the ability to detect fine scale effects of removal that may have been missed at a larger scale. However, it is recommended to conduct a large scale experiment because this would allow for better definition of the broad scale processes not shown by this experiment (Thrush *et al.* 2000). In particular, a large scale removal of *U. lactuca* would result in a significant decline of epifaunal species, the majority of which are grazers. A sudden decline of this group would, in theory, reduce the grazing pressure on the settlement and growth of zoospores and may lead to a high biomass of *U. lactuca* plants in the treated area. This was shown by the results of the *in situ* substrate availability experiment from this study. Exclusion of grazers resulted in a much higher percent cover of *U. lactuca* compared to open treatments. Furthermore, in a laboratory experiment in 1995 on the control of macroalgal blooms at early developmental stages, germination of settled *Enteromorpha* spp. spores and growth of the germlings were reduced by 93-99% in the presence of the grazers *Idotea chelipes* and *Gammarus locusta* (Lotze *et al.* 1999). Therefore, it is important to consider timing and scale of removal for the control of macroalgal blooms.

Sewage diversion and culvert alteration

The Christchurch City Council has proposed, and plans are underway, for the diversion of intertidal sewage outfall into Pegasus Bay off the coast of Christchurch (Moore 2003). This is expected to improve the sand and mudflats in the Estuary, as reported by Arvai *et al.* (2002) in the Fraser River Estuary, Vancouver, (see above). It has been suggested that biomass of *U. lactuca* will reduce by only 20% if the outfall were removed completely, with the greatest reduction close to the causeway and centre of the Estuary (U.R.S. 2001). This was based on the results of ocean modelling work that analysed tidal, wind and outfall locations to predict nutrient status of the waters. Resulting data were combined with modelling studies of *U. lactuca* to predict potential impacts of variation in nutrient levels to biomass of *U. lactuca* in the Estuary. The report also stated that nutrients from the rivers, drains, seepage from the oxidation ponds and within the sediments of the Estuary were sufficient to allow growth to continue and that the majority of the Estuary was unlikely to benefit from the reduced nutrient load by diversion of the sewage outfall.

The predictions of the model mentioned above may well be accurate in respect to nutrient load decrease. However, improvements to the intertidal mudflat ecosystem such as increased dissolved oxygen, decreased heavy metals and increased sediment grain size following diversion of an intertidal outfall (Arvai *et al.* 2002) do not appear to have been included in the model. Improvements such as these lead to increases in abundance and species of benthic fauna in estuarine systems (Arvai *et al.* 2002; Oreja and Salinas 2003; Rogers 2003; Thrush *et al.* 2003). Therefore, I suggest that, along with the improved mudflat ecosystem and increased benthic fauna, grazing pressure upon *U. lactuca* zoospores would increase. The predicted reductions in biomass at the causeway and central Estuary, would act to increase water velocities in these areas. This is because macroalgal mats reduce water velocities in and around the mats (Escartin and Aubrey 1995). This would lead to increased flushing that might further contribute to lower biomass of *U. lactuca*. Furthermore, as sediment grain sizes increase within the Estuary following diversion of the outfall, the potential for the sediments to be a source/sink of nitrogen and phosphorous may be reduced, resulting in decreased *U. lactuca* biomass during spring blooms.

In partnership with the Ihutai Trust, the Christchurch City Council has proposed repair and modification of the central culvert through the causeway separating McCormacks Bay from the main body of the Estuary. This has raised concerns from a variety of ecological, recreational and residential groups that have interests in the highly modified Bay. As a result, an advisory/steering group was formed to facilitate exchange of information between interest

groups and provide a wide knowledge base to aid the Christchurch City Council in the decision making process in relation to the Bay.

The following background on McCormacks Bay is included here for convenience and is repeated from Chapter 2, Section 2.2. McCormacks Bay (Fig 2.1) is a highly modified, shallow, sheltered embayment located on the southern shore of the Estuary (Fig 1.3). The Bay was modified in 1907 when the causeway was built across the tidal mudflats by the Tramway Board. Skylark Island, on the Estuary side, started to erode immediately after this. By 1922 it was reduced to mudflats. In 1933 the causeway was widened for motor traffic (Findlay and Kirk 1988). The Bay is currently connected to the main body of the Estuary by one large and two small culverts passing through the causeway. The causeway and surrounding banks of the Bay act as artificial rocky shores. Prolific beds of *U. lactuca* thrive in the optimal conditions of the Bay. The mudflats are gently sloping from the west past the man-made islands until reaching the main culvert channel (Fig 2.1). The mudflats retain surface water in shallow pools for the duration of low tide. An artificial reef projecting from the left side of the main culvert prevents the eastern area from draining at low tide. The east side of the artificial islands marks the extent of low tide in the western area.

Past modification of McCormacks Bay has resulted in an obvious change in hydrodynamics of the Bay; the majority of water entering and leaving the Bay was restricted to the main culvert. This reduced the amount flushing and freshwater inflow to the Bay from the nearby Heathcote Channel. Evidence from a study in the eutrophic Mondego Estuary, Portugal suggests that this would have favoured growth of *Ulva* spp. (Martins *et al.* 2001). They showed that hydrodynamics was a major factor controlling the occurrence of green macroalgal blooms by analysing data collected between 1993 and 1997 on physico-chemical parameters, biomass and growth of *Enteromorpha* spp. This is comparable to *Ulva* as it is the same genera as *Enteromorpha* (Hayden *et al.* 2003). Martins *et al.* (2001) identified that when sluice gates were closed in winters and springs because of water deficiency in rice fields, low amounts of fresh water entered the Mondego estuarine system in Portugal, salinity remained high, N:P ratios were around $20 \mu\text{mol l}^{-1}$, light penetration increased and current velocities fell. These conditions facilitated growth of spring blooms. *Enteromorpha* spp. also required temps of 10-15°C for germination (Lotze *et al.* 1999). All these conditions are comparable to McCormacks Bay, adding credibility to the hypothesis that McCormacks Bay acts as a favourable nursery site for *U. lactuca*.

I propose that modification to the entire causeway involving opening up the majority of the Bay to the main body of the Estuary (similar to the pre 1907 state) would be expected to significantly reduce the favourable conditions and abundance of *U. lactuca* within the Bay.

This is because the Bay would receive greater freshwater inflow from the Heathcote Channel, and flushing of the Bay would increase (Martins *et al.* 2001). Increased flushing would also result in detached *U. lactuca* plants being flushed from the Bay and out to sea rather than being retained and recycled within the Bay, which adds to its nutritional status. It follows that silt deposition would reduce, possibly resulting in a lowering of the high sediment nitrogen levels in the Bay that is a potential source for promoting *U. lactuca* growth. A reduction of the silt/clay fraction would also promote invertebrate species diversity and abundances as shown by the differences in community assemblages during the removal experiment and general survey; greater diversity and abundance of species was recorded from areas with low silt/clay fraction. Increasing invertebrate species would enhance food availability for shore and nesting birds within the Bay, enhancing the ecological value of the system to residential and recreational groups. In conclusion, the partnership of the Christchurch City Council, the Ihutai Trust and the appointed Advisory/Steering Group are in the process of identifying issues such as these and many more, relating to the culvert's modification. Proposals have been submitted to gather environmental information that will be used to build an ecological profile of the Bay to help determine potential effects of modification on its flora and fauna.

Recommendations and future directions

The findings of my research highlight the necessity of conducting experiments over different spatial and temporal scales, combined with a variety of habitats that exhibit different environmental conditions. In the future, it is recommended that experimental designers consider this so that both local and broad scale processes can be determined. The following is a summary of recommendations that I have considered in light of this research and other observations at the Estuary over the past two years.

- Remove the majority of accumulating macroalgae from McCormacks Bay by hydraulic suction before release of zoospores. This would help lessen spring blooms at the Causeway and Channel sites, as these are the main propagation areas close to the Bay.
- Conserve small areas of macroalgae in the eastern side of the Bay to promote the continued presence of fish species such as yellow-eyed mullet that have been observed to frequent that area.

- Within the main body of the Estuary, remove only large accumulations of *U. lactuca* and conserve areas with low biomass close by to prevent loss of grazers that exert pressure on settled zoospores.
- Conduct regular surveys of physico-chemical variables and community assemblages around the Estuary to determine improvements to the ecosystem following completion of the sewage outfall diversion.
- Conduct transplant experiments of native seagrass *Zostera* sp. to areas devoid of this species as it is known to promote diversity of benthic invertebrates.
- Promote standards to be used in all future research and surveys so that results of comparative studies can be treated confidently as being accurate. A suggested standard is that used by Robertson *et al.* (2002).
- Conduct a large scale removal of *U. lactuca* to compare with the effects reported from the present study.
- Conduct regular removal experiments at a particular site (press disturbance) to compare with the effects of the present (pulse disturbance) study.
- Continue to encourage students, under the supervision of a highly qualified scientist at the University of Canterbury, to be involved in the future research in McCormacks Bay and the main body of the Estuary. This could provide an opportunity for initiating a scholarship fund by interested parties and would provide support and incentive to budding scientists from a broad range of disciplines.

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Appendix 1: Mean number of individual species recorded during the general survey at month 1 (August), 2 (October), 3 (November) and 4 (December).

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| Site | <i>Austrovenus stutchburyi</i> | <i>Macomona liliana</i> | <i>Arthritica bifurca</i> | <i>Mytilus galloprovincialis</i> | <i>Paphies australis</i> | <i>Micrelenchus tenebrosus</i> | <i>Diloma subrostrata</i> | <i>Diloma nigerrima</i> | <i>Zeacumantus subcarinatus</i> | <i>Zeacumantus lutulentus</i> |
|------|------------------------------------|-----------------------------|-------------------------------|--------------------------------------|------------------------------|------------------------------------|-------------------------------|-----------------------------|-------------------------------------|-----------------------------------|
| CW-1 | 190 | 92 | 12 | 0 | 0 | 87 | 23 | 0 | 0 | 0 |
| CW-2 | 369 | 42 | 50 | 1 | 0 | 99 | 35 | 0 | 0 | 0 |
| CW-3 | 117 | 101 | 50 | 0 | 4 | 118 | 14 | 0 | 0 | 0 |
| CW-4 | 103 | 190 | 0 | 1 | 0 | 82 | 0 | 64 | 0 | 0 |
| CH-1 | 155 | 32 | 13 | 0 | 0 | 30 | 24 | 0 | 0 | 0 |
| CH-2 | 204 | 26 | 0 | 0 | 0 | 454 | 0 | 31 | 0 | 0 |
| CH-3 | 162 | 35 | 13 | 1 | 3 | 78 | 29 | 0 | 0 | 0 |
| CH-4 | 230 | 32 | 8 | 1 | 0 | 370 | 0 | 25 | 0 | 0 |
| RB-1 | 12 | 0 | 176 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RB-2 | 8 | 0 | 149 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RB-3 | 3 | 0 | 25 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| RB-4 | 14 | 0 | 35 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| ES-1 | 11 | 5 | 7 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| ES-2 | 61 | 52 | 7 | 0 | 3 | 10 | 0 | 16 | 0 | 0 |
| ES-3 | 16 | 25 | 16 | 0 | 4 | 10 | 0 | 0 | 0 | 0 |
| ES-4 | 50 | 79 | 7 | 0 | 0 | 3 | 0 | 11 | 0 | 0 |
| PS-1 | 20 | 0 | 117 | 0 | 61 | 0 | 0 | 1 | 0 | 0 |
| PS-2 | 101 | 0 | 12 | 0 | 11 | 50 | 0 | 28 | 0 | 0 |
| PS-3 | 16 | 19 | 18 | 0 | 9 | 9 | 16 | 0 | 0 | 0 |
| PS-4 | 94 | 19 | 18 | 0 | 0 | 9 | 0 | 35 | 0 | 0 |
| TS-1 | 46 | 14 | 13 | 0 | 5 | 22 | 4 | 0 | 0 | 0 |
| TS-2 | 62 | 19 | 27 | 0 | 0 | 251 | 0 | 88 | 0 | 0 |
| TS-3 | 45 | 29 | 25 | 0 | 7 | 29 | 3 | 0 | 0 | 0 |
| TS-4 | 68 | 23 | 1 | 1 | 0 | 117 | 0 | 26 | 0 | 0 |
| MB-1 | 5 | 0 | 60 | 0 | 0 | 2 | 0 | 0 | 13 | 6 |
| MB-2 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 39 | 31 |
| MB-3 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 68 | 22 |
| MB-4 | 34 | 0 | 111 | 0 | 0 | 1 | 0 | 4 | 10 | 11 |

Appendix 1: Mean number of individual species recorded during the general survey at month 1 (August), 2 (October), 3 (November) and 4 (December).

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| Site/ month | <i>Potamopyrgus estuarinus</i> | <i>Amphibola crenata</i> | <i>Notoacmea helmsi</i> | <i>Cellana radians</i> | <i>Xymene plebeius</i> | <i>Cominella glandiformis</i> | <i>Anthopleura aureoradiata</i> | <i>Halicarcinus whitei</i> | <i>Helice crassa</i> | <i>Hemigrapsis cranulatus</i> |
|----------------|------------------------------------|------------------------------|-----------------------------|----------------------------|----------------------------|-----------------------------------|-------------------------------------|--------------------------------|--------------------------|-----------------------------------|
| CW-1 | 1 | 0 | 20 | 0 | 0 | 1 | 252 | 3 | 9 | 0 |
| CW-2 | 1 | 0 | 45 | 0 | 0 | 18 | 168 | 3 | 12 | 8 |
| CW-3 | 0 | 0 | 46 | 0 | 0 | 6 | 86 | 1 | 9 | 2 |
| CW-4 | 0 | 0 | 40 | 0 | 0 | 1 | 173 | 2 | 4 | 1 |
| CH-1 | 0 | 0 | 18 | 0 | 3 | 5 | 25 | 0 | 0 | 0 |
| CH-2 | 0 | 0 | 97 | 0 | 2 | 6 | 48 | 0 | 8 | 5 |
| CH-3 | 0 | 0 | 64 | 0 | 3 | 4 | 58 | 0 | 8 | 0 |
| CH-4 | 0 | 0 | 212 | 0 | 3 | 4 | 123 | 1 | 26 | 0 |
| RB-1 | 46 | 31 | 0 | 0 | 0 | 3 | 0 | 5 | 17 | 0 |
| RB-2 | 45 | 58 | 0 | 0 | 0 | 3 | 0 | 0 | 12 | 0 |
| RB-3 | 32 | 37 | 0 | 0 | 0 | 8 | 0 | 7 | 11 | 0 |
| RB-4 | 64 | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 |
| ES-1 | 0 | 52 | 1 | 0 | 0 | 8 | 0 | 0 | 0 | 0 |
| ES-2 | 0 | 41 | 12 | 0 | 0 | 6 | 14 | 0 | 5 | 1 |
| ES-3 | 0 | 54 | 2 | 0 | 0 | 9 | 0 | 0 | 0 | 4 |
| ES-4 | 0 | 1 | 16 | 0 | 0 | 5 | 33 | 1 | 4 | 0 |
| PS-1 | 0 | 250 | 0 | 0 | 0 | 6 | 0 | 6 | 10 | 2 |
| PS-2 | 0 | 32 | 20 | 0 | 0 | 13 | 0 | 3 | 2 | 2 |
| PS-3 | 0 | 39 | 23 | 0 | 0 | 2 | 0 | 6 | 10 | 4 |
| PS-4 | 0 | 55 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 8 |
| TS-1 | 0 | 110 | 8 | 0 | 0 | 2 | 0 | 6 | 8 | 0 |
| TS-2 | 0 | 57 | 23 | 0 | 2 | 9 | 0 | 6 | 12 | 0 |
| TS-3 | 0 | 97 | 9 | 0 | 0 | 4 | 0 | 8 | 12 | 0 |
| TS-4 | 0 | 9 | 50 | 0 | 0 | 15 | 1 | 10 | 29 | 0 |
| MB-1 | 0 | 36 | 3 | 0 | 0 | 3 | 7 | 0 | 17 | 0 |
| MB-2 | 0 | 28 | 11 | 0 | 0 | 7 | 7 | 0 | 9 | 0 |
| MB-3 | 0 | 42 | 21 | 0 | 0 | 5 | 15 | 0 | 7 | 0 |
| MB-4 | 0 | 60 | 1 | 0 | 0 | 7 | 0 | 0 | 5 | 0 |

Appendix 1: Mean number of individual species recorded during the general survey at month 1 (August), 2 (October), 3 (November) and 4 (December).

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| Site/ month | <i>Macrophthalmus hirtipes</i> | <i>Melita awa</i> | <i>Amaurochiton glaucus</i> | <i>Elminius modestus</i> | <i>Scolecoplepides benhami</i> | <i>Boccardia polybranchia</i> | <i>Pectinaria australis</i> | <i>Heteromastus filiformis</i> | <i>Nicon aestuarienus</i> | <i>Scoloplos cylindrifer</i> |
|----------------|------------------------------------|-----------------------|---------------------------------|------------------------------|------------------------------------|-----------------------------------|---------------------------------|------------------------------------|-------------------------------|----------------------------------|
| CW-1 | 3 | 0 | 0 | 0 | 5 | 29 | 4 | 13 | 0 | 6 |
| CW-2 | 6 | 0 | 0 | 0 | 7 | 27 | 4 | 23 | 0 | 5 |
| CW-3 | 4 | 0 | 0 | 0 | 9 | 24 | 6 | 23 | 0 | 3 |
| CW-4 | 2 | 0 | 0 | 0 | 2 | 26 | 2 | 34 | 0 | 12 |
| CH-1 | 2 | 0 | 0 | 3 | 6 | 17 | 8 | 11 | 0 | 20 |
| CH-2 | 0 | 0 | 2 | 0 | 4 | 17 | 10 | 12 | 0 | 27 |
| CH-3 | 0 | 0 | 2 | 19 | 3 | 18 | 10 | 21 | 0 | 10 |
| CH-4 | 0 | 0 | 3 | 17 | 11 | 22 | 11 | 29 | 0 | 28 |
| RB-1 | 0 | 0 | 0 | 0 | 12 | 13 | 0 | 0 | 11 | 0 |
| RB-2 | 0 | 0 | 0 | 0 | 11 | 12 | 0 | 0 | 8 | 0 |
| RB-3 | 0 | 0 | 0 | 0 | 19 | 11 | 0 | 0 | 12 | 0 |
| RB-4 | 0 | 0 | 0 | 0 | 21 | 13 | 0 | 0 | 20 | 0 |
| ES-1 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 21 |
| ES-2 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 |
| ES-3 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 23 |
| ES-4 | 8 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 22 |
| PS-1 | 9 | 0 | 0 | 0 | 7 | 0 | 0 | 2 | 0 | 17 |
| PS-2 | 10 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 20 |
| PS-3 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 3 | 0 | 18 |
| PS-4 | 14 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 21 |
| TS-1 | 7 | 0 | 0 | 0 | 5 | 0 | 0 | 3 | 0 | 9 |
| TS-2 | 13 | 0 | 0 | 0 | 4 | 0 | 0 | 6 | 0 | 8 |
| TS-3 | 7 | 0 | 0 | 0 | 1 | 0 | 0 | 4 | 0 | 5 |
| TS-4 | 20 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 17 |
| MB-1 | 0 | 1 | 0 | 0 | 17 | 16 | 0 | 0 | 6 | 0 |
| MB-2 | 0 | 0 | 0 | 0 | 18 | 16 | 0 | 0 | 6 | 0 |
| MB-3 | 0 | 0 | 0 | 0 | 24 | 3 | 0 | 0 | 11 | 0 |
| MB-4 | 0 | 0 | 0 | 0 | 19 | 2 | 0 | 0 | 17 | 0 |

Appendix 1: Mean number of individual species recorded during the general survey at month 1 (August), 2 (October), 3 (November) and 4 (December).

| Site | <i>Capitella</i> sp. | Sipunculid worm | <i>Ulva</i> spp. | <i>Gracilaria</i> <i>chilensis</i> | <i>Zostera</i> <i>novazelandica</i> |
|------|-------------------------|--------------------|---------------------|---------------------------------------|--|
| CW-1 | 6 | 3 | 2.73 | 0 | 0 |
| CW-2 | 0 | 1 | 1.01 | 0 | 0 |
| CW-3 | 0 | 6 | 14.54 | 0 | 0 |
| CW-4 | 0 | 2 | 17.50 | 0 | 0 |
| CH-1 | 20 | 2 | 0 | 0 | 0 |
| CH-2 | 2 | 0 | 0.84 | 0 | 0 |
| CH-3 | 0 | 0 | 5.66 | 0 | 0 |
| CH-4 | 0 | 0 | 18.37 | 0 | 0 |
| RB-1 | 0 | 14 | 0 | 0 | 0 |
| RB-2 | 17 | 0 | 0 | 0 | 0 |
| RB-3 | 10 | 0 | 0 | 8.39 | 0 |
| RB-4 | 16 | 0 | 14.00 | 0 | 0 |
| ES-1 | 21 | 0 | 0 | 0 | 0 |
| ES-2 | 0 | 3 | 0 | 0 | 0 |
| ES-3 | 5 | 1 | 0 | 0 | 0 |
| ES-4 | 0 | 2 | 3.61 | 0 | 0 |
| PS-1 | 17 | 0 | 0 | 0 | 3.76 |
| PS-2 | 0 | 0 | 0 | 0 | 6.63 |
| PS-3 | 0 | 0 | 0 | 0 | 9.66 |
| PS-4 | 0 | 0 | 0 | 0 | 11.15 |
| TS-1 | 9 | 0 | 0 | 0 | 10.68 |
| TS-2 | 0 | 4 | 0 | 0 | 19.93 |
| TS-3 | 0 | 6 | 0 | 0 | 23.85 |
| TS-4 | 0 | 8 | 1.82 | 0 | 34.00 |
| MB-1 | 0 | 14 | 2.99 | 0 | 0 |
| MB-2 | 15 | 0 | 6.95 | 0 | 0 |
| MB-3 | 13 | 0 | 1.24 | 0 | 0 |
| MB-4 | 13 | 0 | 2.07 | 0 | 0 |

| Size | CW 1 | CW 2 | CW 3 | CW 4 | CH1 | CH2 | CH3 | CH4 | RB1 | RB2 | RB3 | RB4 | ES1 | ES2 | ES3 | ES4 |
|-------------|-------------|-------------|-------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Aug | 35.48 | 37.22 | 39.16 | 29.71 | 40.59 | 41.13 | 43.73 | 37.19 | 22.45 | 20.79 | 18.35 | 20.29 | 34.34 | 27.63 | 25.52 | 30.02 |
| Oct | 24.36 | 31.01 | 34.95 | 36.87 | 20.09 | 29.06 | 39.14 | 43.7 | 20.16 | 20.18 | 19.12 | 24.81 | 26.83 | 28.06 | 30.93 | 31.89 |
| Nov | 33.86 | 39.17 | 39.26 | 37.44 | 39.28 | 41.36 | 41.78 | 41.63 | 20.11 | 26.19 | 21.01 | 24.76 | 32.18 | 26.82 | 28.55 | 29.14 |
| Dec | 42.06 | 33.6 | 33.4 | 33.04 | 43.83 | 47.19 | 43.03 | 43.64 | 22.56 | 24.08 | 23.4 | 24.6 | 27.13 | 28.51 | 27.84 | 26.22 |

| Health | CW 1 | CW 2 | CW 3 | CW 4 | CH1 | CH2 | CH3 | CH4 | RB1 | RB2 | RB3 | RB4 | ES1 | ES2 | ES3 | ES4 |
|---------------|-------------|-------------|-------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Aug | 4.703 | 3.302 | 3.336 | 4.494 | 3.757 | 3.742 | 3.869 | 3.485 | 4.169 | 3.046 | 5.440 | 5.413 | 2.816 | 4.233 | 5.618 | 4.974 |
| Oct | 5.892 | 4.427 | 4.329 | 3.001 | 5.955 | 5.557 | 3.853 | 3.897 | 3.694 | 3.814 | 5.571 | 2.972 | 5.457 | 4.605 | 4.223 | 4.213 |
| Nov | 4.773 | 4.090 | 4.248 | 4.247 | 3.992 | 3.778 | 4.605 | 3.404 | 4.556 | 3.216 | 3.258 | 3.830 | 4.507 | 4.066 | 4.230 | 6.178 |
| Dec | 3.014 | 4.426 | 3.887 | 5.132 | 3.498 | 3.621 | 3.663 | 3.791 | 4.896 | 5.871 | 6.340 | 5.507 | 3.526 | 3.984 | 4.180 | 4.691 |

| Size | PS1 | PS2 | PS3 | PS4 | TS1 | TS2 | TS3 | TS4 | MB1 | MB2 | MB3 | MB4 |
|-------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Aug | 20.52 | 15.98 | 20.4 | 8.87 | 21.7 | 19.76 | 11.43 | 11.13 | 22.02 | 21.2 | 25.32 | 17.37 |
| Oct | 26.67 | 26.93 | 32.52 | 28.08 | 20.21 | 25.44 | 24.15 | 25.43 | 12.57 | 15.79 | 21.29 | 28.5 |
| Nov | 26.87 | 20.56 | 26.15 | 27.52 | 15.04 | 19.21 | 22.84 | 28.91 | 20.2 | 22.54 | 23.87 | 25.68 |
| Dec | 32.76 | 25.11 | 20.95 | 30.43 | 20.89 | 17.39 | 20.51 | 23.87 | 20.76 | 23.58 | 30.97 | 25.47 |

| Health | PS1 | PS2 | PS3 | PS4 | TS1 | TS2 | TS3 | TS4 | MB1 | MB2 | MB3 | MB4 |
|---------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Aug | 6.627 | 7.089 | 7.246 | 6.780 | 7.371 | 6.705 | 6.452 | 6.780 | 6.622 | 7.285 | 5.456 | 4.545 |
| Oct | 7.611 | 5.211 | 4.679 | 8.959 | 6.452 | 5.754 | 6.901 | 4.488 | 6.696 | 5.679 | 6.718 | 5.515 |
| Nov | 4.050 | 6.043 | 3.770 | 5.878 | 5.556 | 7.423 | 6.030 | 4.964 | 5.373 | 5.634 | 6.025 | 5.891 |
| Dec | 3.897 | 5.915 | 5.562 | 2.408 | 7.539 | 6.515 | 5.763 | 5.803 | 6.606 | 7.666 | 4.086 | 6.547 |

| | Pre | Post | Fin | Pre | Post | Fin | Pre | Post | Fin | Pre | Post | Fin |
|----|------|------|-----|------|------|-----|------|------|-----|------|------|-----|
| MB | Cu | Cu | Cu | Cr | Cr | Cr | Ni | Ni | Ni | Zn | Zn | Zn |
| C | 8.7 | 8.1 | 8.6 | 15.5 | 14.5 | 15 | 9.7 | 10.1 | 12 | 72 | 71 | 67 |
| C | 8.9 | 7.5 | 9.2 | 17 | 12.5 | 17 | 9.9 | 9.2 | 12 | 69 | 61.5 | 69 |
| C | 9.5 | 8.3 | 9.1 | 17 | 14.5 | 17 | 10.5 | 11 | 14 | 69.5 | 65 | 66 |
| R | 10 | 8.7 | 10 | 17.5 | 13.5 | 18 | 10 | 9.6 | 13 | 81.5 | 75 | 80 |
| R | 10.4 | 9 | 9.4 | 17 | 15 | 17 | 10.5 | 11.5 | 12 | 78.5 | 75.5 | 72 |
| R | 9.2 | 8.7 | 8.8 | 16.5 | 16 | 17 | 9.8 | 11.5 | 13 | 68 | 69 | 67 |
| MB | Pre | Post | Fin | Pre | Post | Fin | Pre | Post | Fin | | | |
| | Cd | Cd | Cd | Pb | Pb | Pb | As | As | As | | | |
| C | <2 | <2 | <2 | 19.5 | 22 | 18 | 2 | 2 | 2.4 | | | |
| C | <2 | <2 | <2 | 18.5 | 21 | 19 | 2.5 | 2 | 2.7 | | | |
| C | <2 | <2 | <2 | 17.5 | 21.5 | 20 | 2.5 | 2 | 2.4 | | | |
| R | <2 | <2 | <2 | 23 | 23.5 | 26 | 3 | 2.2 | 3.2 | | | |
| R | <2 | <2 | <2 | 19.5 | 24.5 | 20 | 2.5 | 2.1 | 2.5 | | | |
| R | <2 | <2 | <2 | 17 | 23 | 17 | 2.9 | 2.5 | 2.5 | | | |
| MB | Pre | Post | Fin | Pre | Post | Fin | | | | | | |
| | TN | TN | TN | TP | TP | TP | | | | | | |
| C | 870 | 870 | 730 | 472 | 487 | 432 | | | | | | |
| C | 860 | 950 | 850 | 503 | 491 | 487 | | | | | | |
| C | 980 | 820 | 810 | 524 | 491 | 459 | | | | | | |
| R | 1100 | 960 | 940 | 505 | 469 | 472 | | | | | | |
| R | 980 | 970 | 900 | 504 | 498 | 454 | | | | | | |
| R | 900 | 950 | 900 | 491 | 518 | 471 | | | | | | |
| CH | Pre | Post | Fin | Pre | Post | Fin | Pre | Post | Fin | Pre | Post | Fin |
| | Cu | Cu | Cu | Cr | Cr | Cr | Ni | Ni | Ni | Zn | Zn | Zn |
| C | 6 | 5.1 | 4.8 | 22 | 19 | 19 | 13 | 11 | 14 | 58 | 56 | 55 |
| C | 5 | 5.5 | 5 | 20 | 20 | 19 | 12 | 12 | 12 | 57 | 58 | 56 |
| C | 5 | 4.5 | 4.5 | 21 | 18 | 19 | 12 | 11 | 13 | 59 | 55 | 55 |
| R | 5.5 | 5.5 | 5 | 21 | 20 | 19 | 13 | 12 | 13 | 58 | 60 | 55 |
| R | 5 | 5.5 | 4.8 | 21 | 19 | 19 | 13 | 12 | 13 | 58 | 58 | 55 |
| R | 5 | 4.9 | 4.8 | 20 | 18 | 20 | 12 | 11 | 13 | 56 | 56 | 56 |
| CH | Pre | Post | Fin | Pre | Post | Fin | Pre | Post | Fin | | | |
| | Cd | Cd | Cd | Pb | Pb | Pb | As | As | As | | | |
| C | <2 | <2 | <2 | 15 | 11 | 15 | 3.5 | 2.5 | 2.7 | | | |
| C | <2 | <2 | <2 | 14 | 13 | 12 | 3 | 2.5 | 3 | | | |
| C | <2 | <2 | <2 | 14 | 12 | 13 | 3 | 2.5 | 3 | | | |
| R | <2 | <2 | <2 | 15 | 12 | 14 | 3 | 2.5 | 2.8 | | | |
| R | <2 | <2 | <2 | 16 | 11 | 12 | 3 | 2.5 | 2.6 | | | |
| R | <2 | <2 | <2 | 14 | 13 | 13 | 3 | 2.4 | 3.2 | | | |
| CH | Pre | Post | Fin | Pre | Post | Fin | | | | | | |
| | TN | TN | TN | TP | TP | TP | | | | | | |
| C | 460 | 470 | 450 | 403 | 390 | 428 | | | | | | |
| C | 460 | 420 | 430 | 403 | 392 | 444 | | | | | | |
| C | 500 | 450 | 410 | 432 | 368 | 443 | | | | | | |
| R | 550 | 580 | 550 | 385 | 394 | 422 | | | | | | |
| R | 500 | 500 | 480 | 408 | 398 | 427 | | | | | | |
| R | 470 | 460 | 420 | 419 | 346 | 470 | | | | | | |